

This Page Is Inserted by IFW Operations  
and is not a part of the Official Record

## **BEST AVAILABLE IMAGES**

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

**IMAGES ARE BEST AVAILABLE COPY.**

**As rescanning documents *will not* correct images,  
please do not report the images to the  
Image Problem Mailbox.**

THIS PAGE BLANK (USPTO)

10070587

PCT/EP 00 / 08570



Europäisches  
Patentamt

European  
Patent Office

Office européen  
des brevets

REC'D 19 DEC 2000

WIPO

PCT

EP 00/08570

Bescheinigung

Certificate

10 / 070587  
Attestation

Die angehefteten Unterla-  
gen stimmen mit der  
ursprünglich eingereichten  
Fassung der auf dem näch-  
sten Blatt bezeichneten  
europäischen Patentanmel-  
dung überein.

The attached documents  
are exact copies of the  
European patent application  
described on the following  
page, as originally filed.

Les documents fixés à  
cette attestation sont  
conformes à la version  
initialement déposée de  
la demande de brevet  
européen spécifiée à la  
page suivante.

Patentanmeldung Nr. Patent application No. Demande de brevet n°

99118120.7

Der Präsident des Europäischen Patentamts;  
Im Auftrag

For the President of the European Patent Office

Le Président de l'Office européen des brevets  
p.o.

**PRIORITY  
DOCUMENT**  
SUBMITTED OR TRANSMITTED IN  
COMPLIANCE WITH RULE 17.1(a) OR (b)

I.L.C. HATTEN-HECKMAN

DEN HAAG, DEN  
THE HAGUE, 13/12/00  
LA HAYE, LE

**THIS PAGE BLANK (USPTO)**



Europäisches  
Patentamt

European  
Patent Office

Office européen  
des brevets

**Blatt 2 der Bescheinigung**  
**Sheet 2 of the certificate**  
**Page 2 de l'attestation**

Anmeldung Nr.:  
Application no.: 99118120.7  
Demande n°:

Anmeldetag:  
Date of filing: 10/09/99  
Date de dépôt:

Anmelder:  
Applicant(s):  
Demandeur(s):  
Epidauros Biotechnologie AG  
82347 Bernried  
GERMANY

Bezeichnung der Erfindung:  
Title of the invention:  
Titre de l'invention:

Polymorphisms in the human CYP3A4, CYP3A7 and hPXR genes and their use in diagnostic and therapeutic applications

In Anspruch genommene Priorität(en) / Priority(ies) claimed / Priorité(s) revendiquée(s)

Staat:  
State:  
Pays:

Tag:  
Date:  
Date:

Aktenzeichen:  
File no.  
Numéro de dépôt:

Internationale Patentklassifikation:  
International Patent classification:  
Classification internationale des brevets:

C12Q1/68, C12P19/34, C12N9/02, C07K16/18, C12N15/53, A61K38/17, A61P35/00, A01K67/027

Am Anmeldetag benannte Vertragsstaaten:

Contracting states designated at date of filing: AT/BE/CH/CY/DE/DK/ES/FI/FR/GB/GR/IE/IT/LI/LU/MC/NL/PT/SE/

Bemerkungen:  
Remarks:  
Remarques:

**THIS PAGE BLANK (USPTO)**

New EP Application  
EPIDAUROS AG  
Our Ref.: D 2145 EP

EPO-Munich  
60  
10. Sep. 1999

### **Title of the invention**

**Polymorphisms in the human CYP3A4, CYP3A7 and hPXR genes and their use in diagnostic and therapeutic applications**

### **Field of the invention**

The present invention relates generally to means and methods of diagnosing and treating the phenotypic spectrum as well as the overlapping clinical characteristics with several forms of inherited abnormal expression and/or function of the cytochrome P-450 (CYP)3A4 and CYP3A7 and the human pregnane X receptor (hPXR) genes. In particular, the present invention relates to polynucleotides of molecular variant CYP3A4, CYP3A7 and hPXR genes which, for example, are associated with abnormal drug response or individual predisposition to several common cancers caused by environmental carcinogens, and to vectors comprising such polynucleotides. Furthermore, the present invention relates to host cells comprising such polynucleotides or vectors and their use for the production of variant CYP3A4, CYP3A7 and hPXR proteins. In addition, the present invention relates to variant CYP3A4, CYP3A7 and hPXR proteins and antibodies specifically recognizing such proteins. The present invention also concerns transgenic non-human animals comprising the above-described polynucleotide or vectors. Moreover, the present invention relates to methods for identifying and obtaining drug candidates and inhibitors for therapy of disorders related to the malfunction of the CYP3A4, CYP3A7 and hPXR genes as well as to methods of diagnosing the status of such disorders. The present invention furthermore provides pharmaceutical and diagnostic compositions comprising the above-described polynucleotides, vectors, proteins, antibodies, and drugs and inhibitors obtainable by the above-described method. Said compositions are particularly useful for diagnosing and

treating various diseases with drugs that are substrates, inhibitors or modulators of the CYP3A4, CYP3A7 and hPXR genes or their product.

Several documents are cited throughout the text of this specification. Each of the documents cited herein (including any manufacturer's specifications, instructions, etc.) are hereby incorporated herein by reference; however, there is no admission that any document cited is indeed prior art as to the present invention.

### **Background of the invention**

Members of the cytochrome P-450 (CYP) family of hemoproteins metabolise a wide variety of endogenous substrates such as steroid hormones, and of xenobiotics including carcinogens, toxins and drugs(1,2). Of the human CYP proteins, those of the CYP3A subfamily are of a major importance, since collectively, they are by far the most abundant of all the human CYP isoforms. Moreover, their substrate specificity is extremely broad; accordingly, many structurally diverse compounds are, exclusively or to some extent, substrates for CYP3A proteins. Based on the data available it is generally assumed that all CYP3A isoforms have similar substrate spectra; however, limited studies indicate the possibility of differences (3). All CYP3A isoforms are localized in organs of particular importance to drug disposition (gastrointestinal tract, kidney and liver).

At least three functional CYP3A proteins exist in humans. The CYP3A4 monooxygenase is the predominant cytochrome P450 in human liver and small bowel. The protein displays a broad substrate specificity and it metabolises more than 60% of all drugs that are currently in use, including contraceptive steroids, antidepressants, benzodiazepines, immunosuppressive agents, imidazole antimicrobics, and macrolide antibiotics (4,5). In addition, CYP3A4 plays a major role in the protection from environmental toxins. For example, the protein metabolizes aflatoxin B1, which has been implicated in the etiology of liver cancer, which is a major cause of premature death in many areas of Africa and Asia.

Aflatoxin B1 is a mycotoxin produced by species of *Aspergillus*, and human exposure results principally from the ingestion of stored foodstuffs contaminated with the mold. Carcinogenicity is associated with its conversion to 8,9-oxide by the hepatic cytochrome P450-dependent monooxygenase system. Forrester et al. (6) found that the rates of metabolic activation of aflatoxin B1 were highly correlated with the level of proteins of the



CYP3A gene family in the microsomes. Furthermore, Paolini et al. (7) found significant increases in CYP3A in the lungs of rats treated with high doses of beta-carotene. Consequently, it was proposed that correspondingly high levels of CYP3A4 in humans would predispose an individual to cancer risk from the bioactivated tobacco-smoke procarcinogens, thus explaining the cocarcinogenic effect of beta-carotene in smokers. All ~~this implies that individual variation in the CYP3A4 activity could influence the efficacy of a~~ variety of drug therapies as well as the individual predisposition to several major cancers caused by environmental carcinogens.

A considerable variation in the CYP3A4 content and catalytic activity has been, indeed, described in the general population. For example, the metabolic clearance of the gene substrates exhibits a unimodal distribution with up to 20-fold interindividual variability. The activities of the CYP3A4 protein in liver biopsies vary up to 30-fold (8). Furthermore, many common drugs alter the expression levels of the gene (induction or repression) and the extent of this phenomenon is individually variable. The inducers of CYP3A4 expression include commonly used drugs such as the glucocorticoid dexamethasone, the antibiotic rifampicin, and the antimycotic clotrimazole. The inducibility of CYP3A4 expression, combined with the diverse range of substrates, creates a potential for potentially harmful drug interactions involving this isozyme in patients undergoing therapies with multiple drugs.

CYP3A3 is a very closely related isoform to CYP3A4 (>more than 98% cDNA sequence similarity), but it is not known whether this reflects a separate gene product or an allelic variant. By contrast, CYP3A5 is a gene distinct from CYP3A4 and it is expressed polymorphically both in the adult and fetal liver and in the kidney and intestine. In adult Caucasians, the mRNA and the protein were detected in the liver of 10 to 30% of samples, while the protein was detected in the kidney and intestine of 70% of subjects (Ref. (9) and references therein). ~~A point mutation described in the CYP3A5 gene which possibly results~~ in the synthesis of an unstable protein, may account for the polymorphic expression of this enzyme (9). CYP3A7 is the third functional CYP3A isoform. CYP3A7 was originally isolated from a fetal liver but it was subsequently found in 54% of adult livers (10).

Tests to estimate the inducibility and the activity of CYP3A isozymes in an individual patient would be of obvious relevance for the optimization of therapies with drugs which

are their substrates, and for the prevention of the associated side effects. Direct estimates of the activities of CYP3A isozymes in liver biopsies are possible but impracticable for both ethical and cost reasons. The indirect *in vivo* tests of CYP3A4 activity such as the erythromycin breath test or the 6- $\beta$ -hydroxycortisol test pose ethical problems such as the invasive administration of undesirable probe substances and they are obviously unsuited for routine testing. In addition, the lack of correlation between these tests questions their informative value regarding the CYP3A4 activity (11).

A major portion (83%) of the interindividual CYP3A4 variability has been attributed to genetic factors (12). The establishment of a genetic test for the activity of CYP3A4 and of the other CYP3A isozymes should be possible, assuming the prior identification of those factors. Genetic variance affecting the activity and the expression of CYP3A isozymes could be localized in the genes itself, or in one or more of their regulators. A comparison of the three originally published sequences of the best characterized CYP3A gene, CYP3A4, suggested the existence of polymorphisms affecting the amino acid sequence of the CYP3A4 protein (13). Unfortunately, this observation has not been, to our knowledge, confirmed in the general population. More recently, a polymorphism (CYP3A4-W) has been described in the nifedipine-specific response element of the CYP3A4 promoter (14). Its presence associates with a more advanced prostate tumor stage (14). Felix et al. (15) examined this polymorphism in 99 *de novo* and 30 treatment-related leukemias. In all treatment-related cases, there was prior exposure to one or more anticancer drugs metabolized by CYP3A, such as epipodophyllotoxins. These data suggest that individuals with the CYP3A4-W polymorphism may be at increased risk for treatment-related leukemia and that epipodophyllotoxin metabolism by CYP3A4 may contribute to the secondary cancer risk. At present it is unclear if the polymorphism influences the expressivity or inducibility of the CYP3A4 protein. A first published analysis suggests that it has no effect on the basal expression level of CYP3A4 (8). A point mutation was described in the CYP3A5 (9), whereas no mutations have been reported in CYP3A7.

Experiments with amino acid exchanges artificially introduced into the CYP3A4 gene indicate that the function of the family members may be quite sensitive to amino acid exchanges (16-21). Besides amino acid exchanges, silent polymorphisms and those localized in untranslated or intronic sequences also could influence the expression level of

these genes. Alternatively, such polymorphisms could serve as markers for nearby, unidentified polymorphisms. This effect is known as linkage, i.e. defined polymorphisms serve as markers for phenotypes that they are not causative for.

A major breakthrough in the understanding of the CYP3A expression and inducibility took place in 1998 when three research groups independently showed that the expression of CYP3A4 is regulated by a member of the orphan nuclear receptor family termed PXR (pregnane X receptor), or PAR (22-24). Upon treatment with inducers of CYP3A4, PXR binds to the rifampicin/dexamethasone response element in the CYP3A4 promoter as a heterodimer with the 9-cis retinoic acid receptor (RXR). Northern blot analysis detected most abundant expression of hPXR in liver, colon, and small intestine, i.e. in the major organs expressing CYP3A4. The available evidence suggests that human PXR serves as a key transcriptional regulator of the CYP3A4 gene. A recent report describes the induction of CYP3A7 mediated by PXR suggesting that all members of the family may be regulated by this common transcriptional activator (25).

It is clear that naturally occurring mutations, if they exist can have effects on drug metabolism and efficacy of therapies with drugs, in particular in cancer treatment. It is unknown, however, how many of such variations exist, and with what frequency and at what positions in the human CYP3A4, CYP3A7 and hPXR genes.

Accordingly, means and methods for diagnosing and treating a variety of forms of individual drug intolerance and inefficacy of drug therapy which result from CYP3A4, CYP3A7 and/or hPXR gene polymorphisms that interfere e.g., with chemotherapeutic treatment of diseases, in particular cancer, was hitherto not available but are nevertheless highly desirable.

Thus, the technical problem of the present invention is to comply with the needs described above.

The solution to this technical problem is achieved by providing the embodiments characterized in the claims.

### Summary of the Invention

The present invention is based on the finding of novel, so far unknown variations in the nucleotide sequences of the CYP3A4, CYP3A7 and hPXR genes and the population distribution of these alleles. Based upon the knowledge of these novel sequences ~~diagnostic tests and reagents for such tests were designed for the specific detection and~~ genotyping of CYP3A4, CYP3A7 and hPXR alleles in humans, including homozygous as well as heterozygous, frequent as well as rare alleles of the CYP3A4, CYP3A7 and hPXR genes. The determination of the CYP3A4, CYP3A7 and/or hPXR gene allele status of humans with such tests is useful for the optimization of therapies with the numerous substrates of CYP3A4 and CYP3A7. It may also be useful in the determination of the individual predisposition to several common cancers caused by environmental carcinogens.

In a first embodiment, the invention provides polynucleotides of molecular variant CYP3A4, CYP3A7 and hPXR genes and ~~embodiments related thereto~~ such as vectors, host cells, variant CYP3A4, CYP3A7 and hPXR proteins and methods for producing the same.

In yet another embodiment, the invention provides methods for identifying and obtaining drug candidates and inhibitors of CYP3A4, CYP3A7 and hPXR for therapy of disorders related to acquired drug hypo- or hypersensitivity as well as methods of diagnosing the status of such disorders.

In a further embodiment, the invention provides pharmaceutical and diagnostic compositions comprising the above-described polynucleotides, vectors containing the same, proteins, antibodies thereto, and drugs and inhibitors obtainable by the above-described method.

The pharmaceutical and diagnostic compositions, methods and uses of the invention are useful for the diagnosis and treatment of cancer and other diseases the therapy of which is dependent on drug treatment and tolerance. ~~The novel variant forms of CYP3A4, CYP3A7~~ and hPXR genes according to the invention provide the potential for the development of a pharmacodynamic profile of drugs for a given patient.

### Description of the invention

The finding and characterization of variations in the CYP3A4, CYP3A7 and hPXR genes, and diagnostic tests for the discrimination of different CYP3A4, CYP3A7 and hPXR alleles in human individuals provide a very potent tool for improving the therapy of diseases with drugs that are targets of the CYP3A4 or CYP3A7 gene product, and whose metabolism is therefore dependent on CYP3A4 or CYP3A7. The diagnosis of the individual allelic CYP3A4, CYP3A7 and hPXR status permits a more focused therapy, e.g., by opening the possibility to apply individual dose regimens of drugs. It may also be useful as prognostic tool for therapy outcome. Furthermore, diagnostic tests to genotype CYP3A4, CYP3A7 and hPXR, and novel CYP3A4, CYP3A7 and hPXR variants, will not only improve therapy with established drugs and help to correlate genotypes with drug activity or side effects. These tests and sequences also provide reagents for the development of novel inhibitors that specifically modulate the activity of the individual types of CYP3A4, CYP3A7 and hPXR. Expression in yeast, for example, of three allelic cDNAs encoding human liver CYP3A4 and methods for testing the binding properties and catalytic activities of their gene products have been described in (13).

Thus, the present invention provides a novel way to exploit molecular biology and pharmacological research for drug therapy while bypassing their potential detrimental effects which are due to expression of variant CYP3A4, CYP3A7 and hPXR genes.

Accordingly, the invention relates to a polynucleotide selected from the group consisting of:

- (a) polynucleotides comprising a nucleotide sequence encoding the amino acid sequence encodable by a nucleotide sequence of SEQ ID NO: 86, 94 and/or 98;
- (b) polynucleotides comprising a nucleotide sequence encoding a molecular variant of the cytochrome CYP3A4 protein by way of at least one amino acid deletion, addition and/or substitution at an amino acid position corresponding amino acid residue Gly56 in exon 3 of the CYP3A4 gene;
- (c) polynucleotides comprising a nucleotide sequence encoding a molecular variant of the cytochrome CYP3A7 protein by way of at least one amino acid deletion, addition

- and/or substitution, preferably at an amino acid position corresponding amino acid residue Thr 409 in exon 11 of the CYP3A7 gene;
- (d) polynucleotides comprising a nucleotide sequence of any one of SEQ ID NOS: 86, 87, 90, 91, 94, 95 or 108;
  - (e) polynucleotides comprising a nucleotide sequence of any one of SEQ ID NOS: 98, 99, 102, 103, 106 or 107; and
  - (f) polynucleotides comprising a nucleotide sequence encoding a CYP3A4 or CYP3A7 polypeptide or fragment thereof having an epitope comprising an amino acid sequence encoded by the nucleotide sequence of SEQ ID NO: 86 and/or 94.

In the context of the present invention the term "molecular variant" CYP3A4, CYP3A7 or hPXR gene or protein as used herein means that said CYP3A4, CYP3A7 or hPXR gene or protein differs from the wild type CYP3A4, CYP3A7 or hPXR gene or protein by way of nucleotide substitution(s), addition(s) and/or deletion(s). (Genomic sequences of the CYP3A4, CYP3A7 gene are described, for example, Bonk, J Biol Chem 264 (1989), 910-9; Hashimoto, Eur J Biochem 218 (1993), 585-95; Beaune, Proc Natl Acad Sci USA 83 (1986), 8064-8; Malowa, Proc Natl Acad Sci U S A 83 (1986), 5311-5; Accession numbers: M14096, J04449, X12387, M18907. The numbering of the polymorphisms refers to the sequence M14096; for CYP3A7 the reference sequence is described in Komori, J Biochem (Tokyo) 105 (1989), 161-3; Accession number: gi4503232 and for the hPXR gene in Bertilsson, Proc Natl Acad Sci U S A. 95 (1998), 12208-13; Lehmann, J Clin Invest. 102 (1998), 1016-23; Accession numbers: AF061056, AF084645, AF084644. The numbering of the polymorphisms refers to the sequence AF084645). Preferably, said nucleotide substitution(s) result(s) in a corresponding change in the amino acid sequence of the CYP3A4, CYP3A7 or hPXR protein.

In accordance with the present invention, the mode and population distribution of novel so far unidentified genetic variations in the CYP3A4, CYP3A7 and hPXR gene have been analyzed by sequence analysis of relevant regions of the human CYP3A4, CYP3A7 and hPXR genes from many different individuals. It is a well known fact that genomic DNA of individuals, which harbor the individual genetic makeup of all genes, including CYP3A4, CYP3A7 and hPXR can easily be purified from individual blood samples. These individual DNA samples are then used for the analysis of the sequence composition of the CYP3A4,

CYP3A7 and hPXR gene alleles that are present in the individual which provided the blood sample. The sequence analysis was carried out by PCR amplification of relevant regions of the CYP3A4, CYP3A7 and hPXR gene, subsequent purification of the PCR products, followed by automated DNA sequencing with established methods (ABI dyes terminator cycle sequencing).

~~One important parameter that had to be considered in the attempt to determine the~~ individual CYP3A4, CYP3A7 and/or hPXR genotype and identify novel CYP3A4, CYP3A7 or hPXR variants by direct DNA-sequencing of PCR-products from human blood genomic DNA is the fact that each human harbors (usually, with very few abnormal exceptions) two gene copies of each autosomal gene (diploidy). Because of that, great care had to be taken in the evaluation of the sequences to be able to identify unambiguously not only homozygous sequence variations but also heterozygous variations. The details of the different steps in the identification and characterization of novel CYP3A4, CYP3A7 and hPXR gene polymorphisms (homozygous and heterozygous) are described in the examples below.

The mutations in the CYP3A4, CYP3A7 and hPXR genes detected in accordance with the present invention are illustrated in Figure 5 and 6, respectively (indicated by an arrow). The methods of the mutation analysis followed standard protocols and are described in detail in the examples. In general such methods to be used in accordance with the present invention for evaluating the phenotypic spectrum as well as the overlapping clinical characteristics with other forms of drug metabolism and altered tolerance to drugs in patients with mutations in the CYP3A4, CYP3A7 or hPXR gene encompass for example haplotype analysis, single-strand conformation polymorphism analysis (SSCA), PCR and direct sequencing. On the basis of thorough clinical characterization of many patients the phenotypes can then be correlated to these mutations as well as to mutations that had been described earlier.

As is evident to the person skilled in the art this new molecular genetic knowledge can now be used to exactly characterize the genotype of the index patient where a given drug takes an unusual effect and of his family.

Over the past 20 years, genetic heterogeneity has been increasingly recognized as a significant source of variation in drug response. Many scientific communications (Meyer, Ann. Rev. Pharmacol. Toxicol. 37 (1997), 269-296 and West, J. Clin. Pharmacol. 37 (1997), 635-648) have clearly shown that some drugs work better or may even be highly toxic in some patients than in others and that these variations in patient's responses to drugs can be related to molecular basis. This "pharmacogenomic" concept spots correlations between responses to drugs and genetic profiles of patient's (Marshall, Nature Biotechnology, 15 (1997), 954-957; Marshall, Nature Biotechnology, 15 (1997), 1249-1252).

In this context of population variability with regard to drug therapy, pharmacogenomics has been proposed as a tool useful in the identification and selection of patients which can respond to a particular drug without side effects. This identification/selection can be based upon molecular diagnosis of genetic polymorphisms by genotyping DNA from leukocytes in the blood of patient, for example, and characterization of disease (Bertz, Clin. Pharmacokinet. 32 (1997), 210-256; Engel, J. Chromatogr. B. Biomed. Appl. 678 (1996), 93-103). For the providers of health care, such as health maintenance organizations in the US and government public health services in many European countries, this pharmacogenomics approach can represent a way of both improving health care and reducing overheads because there is a large cost to unnecessary therapies, ineffective drugs and drugs with side effects.

The mutations in the variant CYP3A4, CYP3A7 and hPXR genes sometime result in amino acid deletion(s), insertion(s) and in particular in substitution(s) either alone or in combination. It is of course also possible to genetically engineer such mutations in wild type genes or other mutant forms. Methods for introducing such modifications in the DNA sequence of CYP3A4, CYP3A7 or hPXR gene are well known to the person skilled in the art; see, e.g., Sambrook, Molecular Cloning A Laboratory Manual, Cold Spring Harbor Laboratory (1989) N.Y.

In a preferred embodiment of the invention, the above described polynucleotide encodes a variant CYP3A4, CYP3A7 or hPXR protein or fragment thereof, e.g., comprising one or more epitopes of the amino acid sequence encoded by SEQ ID NOS: 86 and 94.



For the investigation of the nature of the alterations in the amino acid sequence of the CYP3A4, CYP3A7 and hPXR proteins computer programs may be used such as BRASMOL that are obtainable from the Internet. Furthermore, folding simulations and computer redesign of structural motifs can be performed using other appropriate computer programs (Olszewski, Proteins 25 (1996), 286-299; Hoffman, Comput. Appl. Biosci. 11 (1995), 675-679). Computers can be used for the conformational and energetic analysis of detailed protein models (Monge, J. Mol. Biol. 247 (1995), 995-1012; Renouf, Adv. Exp. Med. Biol. 376 (1995), 37-45). These analysis can be used for the identification of the influence of a particular mutation on binding and/or metabolism of drugs.

Usually, said amino acid deletion, addition or substitution in the amino acid sequence of the protein encoded by the polynucleotide of the invention is due to one or more nucleotide substitution, insertion or deletion, or any combinations thereof. Preferably said nucleotide substitution, insertion or deletion results in an amino acid substitution of Gly56 to Asp in exon 3 of the CYP3A4 gene and/or Thr409 to Arg in exon 11 of the CYP3A7 gene.

The polynucleotide of the invention may further comprise at least one nucleotide and optionally amino acid deletion, addition and/or substitution other than those specified hereinabove, for example those described in the prior art; e.g.,(13). This embodiment of the present invention allows the study of synergistic effects of the mutations in the CYP3A4, CYP3A7 or hPXR gene on the pharmacological profile of drugs in patients who bear such mutant forms of the gene or similar mutant forms that can be mimicked by the above described proteins. It is expected that the analysis of said synergistic effects provides deeper insights into drug tolerant or sensitive phenotypes of certain forms of cancer and other diseases. From said deeper insight the development of diagnostic and pharmaceutical compositions related to cancer will greatly benefit.

Thus, in a preferred embodiment, the present invention relates to polynucleotides of molecular variant CYP3A4, CYP3A7 and hPXR genes, wherein the nucleotide deletion, addition and/or substitution result in altered expression of the variant CYP3A4, CYP3A7 or hPXR gene compared to the corresponding wild type gene.

The polynucleotide of the invention may be, e.g., DNA, cDNA, genomic DNA, RNA or synthetically produced DNA or RNA or a recombinantly produced chimeric nucleic acid molecule comprising any of those polynucleotides either alone or in combination. Preferably said polynucleotide is part of a vector, particularly plasmids, cosmids, viruses and bacteriophages used conventionally in genetic engineering that comprise a polynucleotide of the invention. Such vectors may comprise further genes such as marker genes which allow for the selection of said vector in a suitable host cell and under suitable conditions.

In a further preferred embodiment of the vector of the invention, the polynucleotide of the invention is operatively linked to expression control sequences allowing expression in prokaryotic or eukaryotic cells. Expression of said polynucleotide comprises transcription of the polynucleotide, preferably into a translatable mRNA. Regulatory elements ensuring expression in eukaryotic cells, preferably mammalian cells, are well known to those skilled in the art. They usually comprise regulatory sequences ensuring initiation of transcription and optionally poly-A signals ensuring termination of transcription and stabilization of the transcript. Additional regulatory elements may include transcriptional as well as translational enhancers. Possible regulatory elements permitting expression in prokaryotic host cells comprise, e.g., the *lac*, *trp* or *tac* promoter in *E. coli*, and examples for regulatory elements permitting expression in eukaryotic host cells are the AOX1 or GAL1 promoter in yeast or the CMV-, SV40-, RSV-promoter (Rous sarcoma virus), CMV-enhancer, SV40-enhancer or a globin intron in mammalian and other animal cells. Beside elements which are responsible for the initiation of transcription such regulatory elements may also comprise transcription termination signals, such as the SV40-poly-A site or the tk-poly-A site, downstream of the polynucleotide. In this context, suitable expression vectors are known in the art such as Okayama-Berg cDNA expression vector pcDV1 (Pharmacia), pCDM8, pRc/CMV, pcDNA1, pcDNA3 (In-vitro gene), pSPORT1 (GIBCO BRL). Preferably, said vector is an expression vector and/or a gene transfer or targeting vector. Expression vectors derived from viruses such as retroviruses, vaccinia virus, adeno-associated virus, herpes viruses, or bovine papilloma virus, may be used for delivery of the polynucleotides or vector of the invention into targeted cell population. Methods which are well known to those skilled in the art can be used to construct recombinant viral vectors; see, for example, the techniques described in Sambrook, Molecular Cloning A Laboratory Manual,

Cold Spring Harbor Laboratory (1989) N.Y. and Ausubel, Current Protocols in Molecular Biology, Green Publishing Associates and Wiley Interscience, N.Y. (1994). Alternatively, the polynucleotides and vectors of the invention can be reconstituted into liposomes for delivery to target cells.

~~The present invention furthermore relates to host cells transformed with a polynucleotide or vector of the invention. Said host cell may be a prokaryotic or eukaryotic cell; see supra. The polynucleotide or vector of the invention which is present in the host cell may either be integrated into the genome of the host cell or it may be maintained extrachromosomally. In this respect, it is also to be understood that the recombinant DNA molecule of the invention can be used for "gene targeting" and/or "gene replacement", for restoring a mutant gene or for creating a mutant gene via homologous recombination; see for example Mouellic, Proc. Natl. Acad. Sci. USA, 87 (1990), 4712-4716; Joyner, Gene Targeting, A Practical Approach, Oxford University Press.~~

The host cell can be any prokaryotic or eukaryotic cell, such as a bacterial, insect, fungal, plant, animal or human cell. Preferred fungal cells are, for example, those of the genus *Saccharomyces*, in particular those of the species *S. cerevisiae*. The term "prokaryotic" is meant to include all bacteria which can be transformed or transfected with a polynucleotide for the expression of a variant CYP3A4, CYP3A7 or hPXR protein or fragment thereof. Prokaryotic hosts may include gram negative as well as gram positive bacteria such as, for example, *E. coli*, *S. typhimurium*, *Serratia marcescens* and *Bacillus subtilis*. A polynucleotide coding for a mutant form of CYP3A4, CYP3A7 and hPXR variant proteins can be used to transform or transfect the host using any of the techniques commonly known to those of ordinary skill in the art. Methods for preparing fused, operably linked genes and expressing them in bacteria or animal cells are well-known in the art (Sambrook, supra). The genetic constructs and methods described therein can be utilized for expression of variant CYP3A4, CYP3A7 and hPXR proteins in, e.g., prokaryotic hosts. ~~In general, expression vectors containing promoter sequences which facilitate the efficient~~ transcription of the inserted polynucleotide are used in connection with the host. The expression vector typically contains an origin of replication, a promoter, and a terminator, as well as specific genes which are capable of providing phenotypic selection of the transformed cells. The transformed prokaryotic hosts can be grown in fermentors and cultured according to techniques known in the art to achieve optimal cell growth. The

proteins of the invention can then be isolated from the grown medium, cellular lysates, or cellular membrane fractions. The isolation and purification of the microbially or otherwise expressed polypeptides of the invention may be by any conventional means such as, for example, preparative chromatographic separations and immunological separations such as those involving the use of monoclonal or polyclonal antibodies.

Thus, in a further embodiment the invention relates to a method for the production of variant CYP3A4, CYP3A7 and hPXR proteins and fragments thereof comprising culturing a host cell as defined above under conditions allowing the expression of the protein and recovering the produced protein or fragment from the culture.

In another embodiment the present invention relates to a method for producing cells capable of expressing a variant CYP3A4, CYP3A7 and/or hPXR gene comprising genetically engineering cells with the polynucleotide or with the vector of the invention. The cells obtainable by the method of the invention can be used, for example, to test drugs according to the methods described in Sambrook, Fritsch, Maniatis (1989), Molecular cloning: a laboratory manual, Cold Spring Harbour Laboratory press, Cold Spring Harbour; Peyronneau, Eur. J. Biochem. 218 (1993), 355-361; Yamazaki, Carcinogenesis, 16 (1995), 2167-2170. Furthermore, the cells can be used to study known drugs and unknown derivatives thereof for their ability to complement loss of drug efficacy caused by mutations in the CYP3A4, CYP3A7 or hPXR gene. For these embodiments the host cells preferably lack a wild type allele, preferably both alleles of the CYP3A4, CYP3A7 and/or hPXR gene and/or have at least one mutated from thereof. Alternatively, strong overexpression of a mutated allele over the normal allele and comparison with a recombinant cell line overexpressing the normal allele at a similar level may be used as a screening and analysis system. The cells obtainable by the above-described method may also be used for the screening methods referred to herein below.

Furthermore, the invention relates to variant CYP3A4, CYP3A7 and hPXR proteins and fragments thereof encoded by a polynucleotide according to the invention or obtainable by the above-described methods or from cells produced by the method described above. In this context it is also understood that the variant CYP3A4, CYP3A7 and hPXR proteins according to the invention may be further modified by conventional methods known in the

art. By providing the variant CYP3A4, CYP3A7 and hPXR proteins according to the present invention it is also possible to determine the portions relevant for their biological activity or inhibition of the same.

The present invention furthermore relates to antibodies specifically recognizing a variant CYP3A4, CYP3A7 or hPXR protein according to the invention. Advantageously, the antibody specifically recognizes an epitope containing one or more amino acid substitution(s) as defined above

Antibodies against the variant CYP3A4, CYP3A7 or hPXR protein of the invention can be prepared by well known methods using a purified protein according to the invention or a (synthetic) fragment derived therefrom as an antigen. Monoclonal antibodies can be prepared, for example, by the techniques as originally described in Köhler and Milstein, Nature 256 (1975), 495, and Galfré, Meth. Enzymol. 73 (1981), 3, which comprise the fusion of mouse myeloma cells to spleen cells derived from immunized mammals. The antibodies can be monoclonal antibodies, polyclonal antibodies or synthetic antibodies as well as fragments of antibodies, such as Fab, Fv or scFv fragments etc. Furthermore, antibodies or fragments thereof to the aforementioned polypeptides can be obtained by using methods which are described, e.g., in Harlow and Lane "Antibodies, A Laboratory Manual", CSH Press, Cold Spring Harbor, 1988. These antibodies can be used, for example, for the immunoprecipitation and immunolocalization of the variant CYP3A4, CYP3A7 and hPXR proteins of the invention as well as for the monitoring of the presence of such variant CYP3A4, CYP3A7 and hPXR proteins, for example, in transgenic organisms, and for the identification of compounds interacting with the proteins according to the invention. For example, surface plasmon resonance as employed in the BIAcore system can be used to increase the efficiency of phage antibodies which bind to an epitope of the protein of the invention (Schier, Human Antibodies Hybridomas 7 (1996), 97-105; Malmberg, J. Immunol. Methods 183 (1995), 7-13).

Furthermore, the present invention relates to nucleic acid molecules which represent or comprise the complementary strand of any of the above described polynucleotides or a part thereof, thus comprising at least one nucleotide difference compared to the corresponding wild type CYP3A4, CYP3A7 and hPXR gene nucleotide sequences specified by the above described nucleotide substitutions, deletions and additions. Such a

molecule may either be a deoxyribonucleic acid or a ribonucleic acid. Such molecules comprise, for example, antisense RNA. These molecules may furthermore be linked to sequences which when transcribed code for a ribozyme thereby producing a ribozyme which specifically cleaves transcripts of polynucleotides according to the invention.

~~Furthermore, the present invention relates to a vector comprising a nucleic acid molecule~~ according to the invention. Examples for such vectors are described above. Preferably, the nucleic acid molecule present in the vector is operatively linked to regulatory elements permitting expression in prokaryotic or eukaryotic host cells; see supra.

The present invention also relates to a method for the production of a transgenic non-human animal, preferably transgenic mouse, comprising introduction of a polynucleotide or vector of the invention into a germ cell, an embryonic cell, stem cell or an egg or a cell derived therefrom. The non-human animal can be used in accordance with the method of the invention described below and may be a non-transgenic healthy animal, or may have a disorder, preferably a disorder caused by at least one mutation in the CYP3A4, CYP3A7 and/or hPXR gene. Such transgenic animals are well suited for, e.g., pharmacological studies of drugs in connection with variant forms of the above described variant CYP3A4, CYP3A7 and hPXR proteins, since these proteins or at least their functional domains are conserved between species in higher eukaryotes, particularly in mammals. Production of transgenic embryos and screening of those can be performed, e.g., as described by A. L. Joyner Ed., Gene Targeting, A Practical Approach (1993), Oxford University Press. The DNA of the embryos can be analyzed using, e.g., Southern blots with an appropriate probe.

The invention also relates to transgenic non-human animals such as transgenic mouse, rats, hamsters, dogs, monkeys, rabbits, pigs, C. elegans and fish such as torpedo fish comprising a polynucleotide or vector of the invention or obtained by the method described above, preferably wherein said polynucleotide or vector is stably integrated into the genome of said non-human animal, preferably such that the presence of said polynucleotide or vector leads to the expression of the variant CYP3A4, CYP3A7 and/or hPXR gene of the invention. It may have one or several copies of the same or different polynucleotides of the variant CYP3A4, CYP3A7 or hPXR gene. This animal has

numerous utilities, including as a research model for drug tolerability and therefore, presents a novel and valuable animal in the development of therapies, treatment, etc. for diseases caused by deficiency or failure of drug metabolism in the cell. Accordingly, in this instance, the mammal is preferably a laboratory animal such as a mouse or rat.

~~Preferably, the transgenic non-human animal of the invention further comprises at least one inactivated wild type allele of the CYP3A4, CYP3A7 and/or hPXR gene. This embodiment allows for example the study of the interaction of various variant forms of CYP3A4, CYP3A7 and hPXR proteins. It might be also desirable to inactivate CYP3A4, CYP3A7 and/or hPXR gene expression or function at a certain stage of development and/or life of the transgenic animal. This can be achieved by using, for example, tissue specific, developmental and/or cell regulated and/or inducible promoters which drive the expression of, e.g., an antisense or ribozyme directed against the RNA transcript of the CYP3A4, CYP3A7 or hPXR gene; see also supra. A suitable inducible system is for example tetracycline-regulated gene expression as described, e.g., by Gossen and Bujard (Proc. Natl. Acad. Sci. 89 USA (1992), 5547-5551) and Gossen et al. (Trends Biotech. 12 (1994), 58-62). Similar, the expression of the variant CYP3A4, CYP3A7 and hPXR gene may be controlled by such regulatory elements.~~

With the variant CYP3A4, CYP3A7 and hPXR polynucleotides and proteins and vectors of the invention, it is now possible to study *in vivo* and *in vitro* the efficiency of drugs in relation to particular mutations in the CYP3A4, CYP3A7 or hPXR gene of a patient and the affected phenotype. Furthermore, the variant CYP3A4, CYP3A7 and hPXR proteins of the invention can be used to determine the pharmacological profile of drugs and for the identification and preparation of further drugs which may be more effective for the treatment of, e.g., cancer, in particular for the amelioration of certain phenotypes caused by the respective mutations such as those described above.

Thus, a particular object of the present invention concerns drug/pro-drug selection and formulation of pharmaceutical compositions for the treatment of diseases which are amenable to chemotherapy taking into account the polymorphism of the variant form of the CYP3A4, CYP3A7 or hPXR gene that cosegregates with the affected phenotype of the patient to be treated. This allows the safe and economic application of drugs which for

example were hitherto considered not appropriate for therapy of, e.g., cancer due to either their side effects in some patients and/or their unreliable pharmacological profile with respect to the same or different phenotype(s) of the disease. The means and methods described herein can be used, for example, to improve dosing recommendations and allows the prescriber to anticipate necessary dose adjustments depending on the considered patient group.

In a further embodiment the present invention relates to a method of identifying and obtaining an CYP3A4, CYP3A7 or hPXR inhibitor capable of modulating the activity of a molecular variant of the CYP3A4, CYP3A7 or hPXR gene or its gene product comprising the steps of

- (a) contacting the variant CYP3A4, CYP3A7 or hPXR protein or a cell expressing a molecular variant gene comprising a polynucleotide of the invention in the presence of components capable of providing a detectable signal in response to drug metabolism, with a compound to be screened under conditions to permit CYP3A4 or CYP3A7-mediated drug metabolism, and
- (b) detecting the presence or absence of a signal or increase of a signal generated from the metabolized drug, wherein the presence or increase of the signal is indicative for a putative inhibitor.

The term "compound" in a method of the invention includes a single substance or a plurality of substances which may or may not be identical.

Said compound(s) may be chemically synthesized or produced via microbial fermentation but can also be comprised in, for example, samples, e.g., cell extracts from, e.g., plants, animals or microorganisms. Furthermore, said compounds may be known in the art but hitherto not known to be useful as an inhibitor, respectively. The plurality of compounds may be, e.g., added to the culture medium or injected into a cell or non-human animal of the invention.

If a sample containing (a) compound(s) is identified in the method of the invention, then it is either possible to isolate the compound from the original sample identified as containing the compound, in question or one can further subdivide the original sample, for example, if it consists of a plurality of different compounds, so as to reduce the number of different substances per sample and repeat the method with the subdivisions of the original sample.



It can then be determined whether said sample or compound displays the desired properties, for example, by the methods described herein or in the literature (e.g. (13) and Lehmann, J Clin Invest 102 (1998), 1016-23). Depending on the complexity of the samples, the steps described above can be performed several times, preferably until the sample identified according to the method of the invention only comprises a limited number of or only one substance(s). Preferably said sample comprises substances of similar chemical and/or physical properties, and most preferably said substances are identical. The methods of the present invention can be easily performed and designed by the person skilled in the art, for example in accordance with other cell based assays described in the prior art or by using and modifying the methods as described herein. Furthermore, the person skilled in the art will readily recognize which further compounds and/or enzymes may be used in order to perform the methods of the invention, for example, enzymes, if necessary, that convert a certain compound into the precursor which in turn represents a substrate for the CYP3A4 or CYP3A7 protein. Such adaptation of the method of the invention is well within the skill of the person skilled in the art and can be performed without undue experimentation.

Suitable assays which can be employed in accordance with the present invention are described, for example, in Hashimoto, Eur J Biochem 218 (1993), 585-95 wherein transfection assays with chimeric CYP3A4 genes in HepG2 cells are described. Similarly, the variant CYP3A4, CYP3A7 and/or hPXR genes can be expressed or co-expressed in HepG2 cells and analyzed for their transcriptional activity and catalytic properties of CYP3A4 or CYP3A7. Such an assay can also be used for studying the catalytic properties of the CYP3A4 and CYP3A7 on its substrates such as steroids (testosterone, progesterone, androstenedione, cortisol,  $17\beta$ -oestradiol,  $17\alpha$ -ethynyloestradiol), antibiotics (erythromycin), immunosuppressive (cyclosporine A), benzodiazepine (midazolam), benzothiazepine derivatives (diltiazem, triazolam), and nifedipine. In particular, such tests are useful to add in predicting whether a given drug will interact in an individual carrying the respective variant CYP3A4, CYP3A7 and/or hPXR gene. A suitable expression system which can be employed in accordance with above described methods of the present invention is also described in (22). In addition heterologous expression systems such as yeast can be used in order to study the stability, binding properties and catalytic activities of the gene products of the variant CYP3A4, CYP3A7 and hPXR genes compared to the

corresponding wild type gene product. As mentioned before, the molecular variant CYP3A4, CYP3A7 and hPXR genes and their gene products, particularly when employed in the above described methods, can be used for pharmacological and toxicological studies of the metabolism of drugs. Preferred drugs to be tested in accordance with the methods of the present invention comprise those described above and include, but are not limited to nifedipine, erythromycin, troleandomycin, quinidine, cyclosporin A, 17  $\alpha$ -ethynylestradiol, lidocaine, diltiazem, dexamethasone, RU486, see also supra.

Compounds which can be used in accordance with the present invention include peptides, proteins, nucleic acids, antibodies, small organic compounds, ligands, peptidomimetics, PNAs and the like. Said compounds can also be functional derivatives or analogues of known drugs such as from those described above. Methods for the preparation of chemical derivatives and analogues are well known to those skilled in the art and are described in, for example, Beilstein, Handbook of Organic Chemistry, Springer edition New York Inc., 175 Fifth Avenue, New York, N.Y. 10010 U.S.A. and Organic Synthesis, Wiley, New York, USA. Furthermore, said derivatives and analogues can be tested for their effects according to methods known in the art or as described. Furthermore, peptide mimetics and/or computer aided design of appropriate drug derivatives and analogues can be used, for example, according to the methods described below. Such analogs comprise molecules having as the basis structure of known CYP3A4 and CYP3A7-substrates and/or inhibitors and/or modulators; see infra.

Appropriate computer programs can be used for the identification of interactive sites of a putative inhibitor and the CYP3A4, CYP3A7 or hPXR protein of the invention by computer assistant searches for complementary structural motifs (Fassina, Immunomethods 5 (1994), 114-120). Further appropriate computer systems for the computer aided design of protein and peptides are described in the prior art, for example, in Berry, Biochem. Soc. Trans. 22 (1994), 1033-1036; Wodak, Ann. N. Y. Acad. Sci. 501 (1987), 1-13; Pabo, Biochemistry 25 (1986), 5987-5991. The results obtained from the above-described computer analysis can be used in combination with the method of the invention for, e.g., optimizing known inhibitors. Appropriate peptidomimetics and other inhibitors can also be identified by the synthesis of peptidomimetic combinatorial libraries through successive chemical modification and testing the resulting compounds, e.g., according to the methods described herein. Methods for the generation and use of peptidomimetic combinatorial libraries are described in the prior art, for example in Ostresh, Methods in Enzymology 267

(1996), 220-234 and Dorner, Bioorg. Med. Chem. 4 (1996), 709-715. Furthermore, the three-dimensional and/or crystallographic structure of inhibitors and the CYP3A4, CYP3A7 or hPXR protein of the invention can be used for the design of peptidomimetic drugs (Rose, Biochemistry 35 (1996), 12933-12944; Rutenberg, Bioorg. Med. Chem. 4 (1996), 1545-1558).

In summary, the present invention provides methods for identifying and obtaining compounds which can be used in specific doses for the treatment of specific forms of diseases, e.g., cancer the chemotherapy of which is complicated by malfunctions of the CYP3A4, CYP3A7 or hPXR gene often resulting in an altered activity or level of drug metabolism or sensitive phenotype.

In a preferred embodiment of the method of the invention said cell is a cell of or, obtained by the method of the invention or is comprised in the above-described transgenic non-human animal.

In a further embodiment the present invention relates to a method of identifying and obtaining an CYP3A4, CYP3A7 or hPXR inhibitor capable of modulating the activity of a molecular variant of the CYP3A4, CYP3A7 or hPXR gene or its gene product comprising the steps of

- (a) contacting the variant CYP3A4, CYP3A7 or hPXR protein of the invention with a first molecule known to be bound by CYP3A4, CYP3A7 or hPXR protein to form a first complex of said protein and said first molecule;
- (b) contacting said first complex with a compound to be screened; and
- (c) measuring whether said compound displaces said first molecule from said first complex.

Advantageously, in said method said measuring step comprises measuring the formation of a second complex of said protein and said inhibitor candidate. Preferably, said measuring step comprises measuring the amount of said first molecule that is not bound to said protein.

In a particularly preferred embodiment of the above-described method of said first molecule is nifedipine, rifampicine or corticosterone. Furthermore, it is preferred that in the method of the invention said first molecule is labeled, e.g., with a radioactive or fluorescent label.

~~In a still further embodiment the present invention relates to a method of diagnosing a disorder related to the presence of a molecular variant CYP3A4, CYP3A7 or hPXR gene or susceptibility to such a disorder comprising~~

- (a) determining the presence of a polynucleotide of the invention in a sample from a subject; and/or
- (b) determining the presence of a variant form of CYP3A4, CYP3A7 or hPXR protein, for example, with the antibody of the invention.

~~In accordance with this embodiment of the present invention, the method of testing the status of a disorder or susceptibility to such a disorder can be effected by using a polynucleotide or a nucleic acid molecule of the invention, e.g., in the form of a Southern or Northern blot or *in situ* analysis. Said nucleic acid sequence may hybridize to a coding region of either of the genes or to a non-coding region, e.g. intron. In the case that a complementary sequence is employed in the method of the invention, said nucleic acid molecule can again be used in Northern blots. Additionally, said testing can be done in conjunction with an actual blocking, e.g., of the transcription of the gene and thus is expected to have therapeutic relevance. Furthermore, a primer or oligonucleotide can also be used for hybridizing to one of the above-mentioned CYP3A4, CYP3A7 or hPXR genes or corresponding mRNAs. The nucleic acids used for hybridization can, of course, be conveniently labeled by incorporating or attaching, e.g., a radioactive or other marker. Such markers are well known in the art. The labeling of said nucleic acid molecules can be effected by conventional methods.~~

~~Additionally, the presence or expression of variant CYP3A4, CYP3A7 and hPXR genes can be monitored by using a primer pair that specifically hybridizes to either of the corresponding nucleic acid sequences and by carrying out a PCR reaction according to standard procedures. Specific hybridization of the above mentioned probes or primers preferably occurs at stringent hybridization conditions. The term "stringent hybridization conditions" is well known in the art; see, for example, Sambrook et al., "Molecular Cloning,~~

A Laboratory Manual" second ed., CSH Press, Cold Spring Harbor, 1989; "Nucleic Acid Hybridisation, A Practical Approach", Hames and Higgins eds., IRL Press, Oxford, 1985. Furthermore, the mRNA, cRNA, cDNA or genomic DNA obtained from the subject may be sequenced to identify mutations which may be characteristic fingerprints of mutations in the CYP3A4, CYP3A7 and hPXR gene. The present invention further comprises methods wherein such a fingerprint may be generated by RFLPs of DNA or RNA obtained from the subject, optionally the DNA or RNA may be amplified prior to analysis, the methods of which are well known in the art. RNA fingerprints may be performed by, for example, digesting an RNA sample obtained from the subject with a suitable RNA-Enzyme, for example RNase T<sub>1</sub>, RNase T<sub>2</sub> or the like or a ribozyme and, for example, electrophoretically separating and detecting the RNA fragments as described above.

Further modifications of the above-mentioned embodiment of the invention can be easily devised by the person skilled in the art, without any undue experimentation from this disclosure; see, e.g., the examples. An additional embodiment of the present invention relates to a method wherein said determination is effected by employing an antibody of the invention or fragment thereof. The antibody used in the method of the invention may be labeled with detectable tags such as a histidine flags or a biotin molecule.

In a preferred embodiment of the present invention, the above described methods comprise PCR, ligase chain reaction, restriction digestion, direct sequencing, nucleic acid amplification techniques, hybridization techniques or immunoassays (Sambrook et al., loc. cit. CSH cloning, Harlow and Lane loc. cit. CSH antibodies).

In a preferred embodiment of the method of the present invention said disorder is cancer.

In a further embodiment of the above-described method, a further step comprising administering to the subject a medicament to abolish or alleviate said variations in the CYP3A4, CYP3A7 or hPXR gene in accordance with all applications of the method of the invention allows treatment of a given disease before the onset of clinical symptoms due to the phenotype response caused by the CYP3A4, CYP3A7 or hPXR gene.

In a preferred embodiment of the method of the invention said medicament are chemotherapeutic agents such as substrates of CYP3A4: paclitaxen (Eur J Drug Metab

Pharmacokinet 23 (1998), 417-24), tamoxifen and toremifene (Drug Metab Dispos 27(1999), 681-8; Clin Pharmacol Ther 64 (1998), 648-54; Clin Pharmacol Ther 57 (1995), 628-35), trofosfamide (Cancer Chemother Pharmacol 44(1999), 327-334), cyclophosphamide and ifosfamide (Drug Metab Dispos 27 (1999), 655-66; Cancer Res 58 (1998), 4391-401; Br J Clin Pharmacol 40 (1995), 523-30), taxotere (Pharmacogenetics 8 (1998), 391-401; Clarke, Clin Pharmacokinet 36 (1999), 99-114).

---

In another preferred embodiment of the above-described methods, said method further comprises introducing

- (i) a functional and expressible wild type CYP3A4, CYP3A7 or hPXR gene or
- (ii) a nucleotide acid molecule or vector of the invention into cells.

In this context and as used throughout this specification, "functional" CYP3A4, CYP3A7 and hPXR gene means a gene wherein the encoded protein having part or all of the primary structural conformation of the wild type CYP3A4, CYP3A7 and hPXR protein, i.e. possessing the biological property of metabolizing drugs and controlling the CYP3A4, CYP3A7 gene, respectively. This embodiment of the present invention is suited for therapy of cancer in particular in humans. Detection of the expression of a variant CYP3A4 or CYP3A7 and/or hPXR gene would allow the conclusion that said expression is interrelated to the generation or maintenance of a corresponding phenotype of the disease. Accordingly, a step would be applied to reduce the expression level to low levels or abolish the same. This can be done, for example, by at least partial elimination of the expression of the mutant gene by biological means, for example, by the use of ribozymes, antisense nucleic acid molecules, intracellular antibodies or the above described inhibitors against the variant forms of these CYP3A4, CYP3A7 and/or hPXR proteins. Furthermore, pharmaceutical products may be developed that reduce the expression levels of the corresponding mutant proteins and genes.

---

In a further embodiment the invention relates to a method for the production of a pharmaceutical composition comprising the steps of any one of the above described methods and synthesizing and/or formulating the compound identified in step (b) or a derivative or homologue thereof in a pharmaceutically acceptable form. The therapeutically useful compounds identified according to the method of the invention may be formulated

and administered to a patient as discussed above. For uses and therapeutic doses determined to be appropriate by one skilled in the art see *infra*.

Furthermore, the present invention relates to a method for the preparation of a pharmaceutical composition comprising the steps of the above-described methods; and ~~formulating a drug or pro-drug in the form suitable for therapeutic application and~~ preventing or ameliorating the disorder of the subject diagnosed in the method of the invention.

Drugs or pro-drugs after their *in vivo* administration are metabolized in order to be eliminated either by excretion or by metabolism to one or more active or inactive metabolites (Meyer, J. Pharmacokinet. Biopharm. 24 (1996), 449-459). Thus, rather than using the actual compound or inhibitor identified and obtained in accordance with the methods of the present invention a corresponding formulation as a pro-drug can be used which is converted into its active in the patient. Precautionary measures that may be taken for the application of pro-drugs and drugs are described in the literature; see, for review, Ozama, J. Toxicol. Sci. 21 (1996), 323-329).

In a preferred embodiment of the method of the present invention said drug or prodrug is a derivative of a medicament as defined hereinbefore.

In a still further embodiment the present invention relates to an inhibitor identified or obtained by the method described hereinbefore. Preferably, the inhibitor binds specifically to the variant CYP3A4, CYP3A7 or hPXR protein of the invention. The antibodies, nucleic acid molecules and inhibitors of the present invention preferably have a specificity at least substantially identical to binding specificity of the natural ligand or binding partner of the CYP3A4, CYP3A7 or hPXR protein of the invention. An antibody or inhibitor can have a binding affinity to the CYP3A4, CYP3A7 or hPXR protein of the invention of at least  $10^5 \text{ M}^{-1}$ , preferably higher than  $10^7 \text{ M}^{-1}$  and advantageously up to  $10^{10} \text{ M}^{-1}$  in case CYP3A4, CYP3A7 or hPXR activity should be repressed. Hence, in a preferred embodiment, a suppressive antibody or inhibitor of the invention has an affinity of at least about  $10^{-7} \text{ M}$ , preferably at least about  $10^{-9} \text{ M}$  and most preferably at last about  $10^{-11} \text{ M}$ .

Furthermore, the present invention relates to the use of an oligo- or polynucleotide for the detection of a polynucleotide of the invention and/or for genotyping of corresponding individual CYP3A4, CYP3A7 or hPXR alleles. Preferably, said oligo- or polynucleotide is a polynucleotide or a nucleic acid molecule of the invention described before.

In a particular preferred embodiment said oligonucleotide is about 15 to 50, preferably 20 to 40, more preferably 20 to 30 nucleotides in length and comprises the nucleotide sequence of any one of SEQ ID NOS: 1 to 107 or a complementary sequence.

Hence, in a still further embodiment, the present invention relates to a primer or probe consisting of an oligonucleotide as defined above. In this context, the term "consisting of" means that the nucleotide sequence described above and employed for the primer or probe of the invention does not have any further nucleotide sequences of the CYP3A4, CYP3A7 or hPXR gene immediately adjacent at its 5' and/or 3' ends. However, other moieties such as labels, e.g., biotin molecules, histidin flags, antibody fragments, colloidal gold, etc., as well as nucleotide sequences which do not correspond to the CYP3A4, CYP3A7 or hPXR gene may be present in the primer and probes of the present invention. Furthermore, it is also possible to use the above described particular nucleotide sequences and to combine them with other nucleotide sequences derived from the CYP3A4, CYP3A7 or hPXR gene wherein these additional nucleotide sequences are interspersed with moieties other than nucleic acids or wherein the nucleic acid does not correspond to nucleotide sequences of the CYP3A4, CYP3A7 or hPXR gene. Furthermore, it is evident to the person skilled in the art that the oligonucleotide can be modified, for example, by thio-phosphate-backbones and/or base analogs well known in the art (Flanagan, Proc. Natl. Acad. Sci. USA 96 (1999), 3513-8; Witters, Breast Cancer Res. Treat. 53 (1999), 41-50; Hawley, Antisense Nucleic Acid Drug Dev. 9 (1999), 61-9; Peng-Ho, Brain Res. Mol. Brain Res. 62 (1998), 1-11; Spiller, Antisense Nucleic Acid Drug Dev. 8 (1998), 281-93; Zhang, J. Pharmacol. Exp. Ther. 278 (1996), 971-9; Shoji, Antimicrob. Agents Chemother. 40 (1996), 1670-5; Crooke, J. Pharmacol. Exp. Ther. 277 (1996), 923-37).

In addition, the present invention relates to the use of an antibody or a substance capable of binding specifically to the gene product of an CYP3A4, CYP3A7 or hPXR gene for the



detection of the variant CYP3A4, CYP3A7 or hPXR protein of the invention, the expression of a molecular variant CYP3A4, CYP3A7 or hPXR gene comprising a polynucleotide of the invention and/or for distinguishing CYP3A4, CYP3A7 and hPXR alleles comprising a polynucleotide of the invention.

~~Moreover, the present invention relates to a composition, preferably pharmaceutical~~ composition comprising the antibody, the nucleic acid molecule, the vector or the inhibitor of the present invention, and optionally a pharmaceutically acceptable carrier. These pharmaceutical compositions comprising, e.g., the inhibitor or pharmaceutically acceptable salts thereof may conveniently be administered by any of the routes conventionally used for drug administration, for instance, orally, topically, parenterally or by inhalation. Acceptable salts comprise acetate, methylester, HCl, sulfate, chloride and the like. The compounds may be administered in conventional dosage forms prepared by combining the drugs with standard pharmaceutical carriers according to conventional procedures. These procedures may involve mixing, granulating and compressing or dissolving the ingredients as appropriate to the desired preparation. It will be appreciated that the form and character of the pharmaceutically acceptable character or diluent is dictated by the amount of active ingredient with which it is to be combined, the route of administration and other well-known variables. The carrier(s) must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not deleterious to the recipient thereof. The pharmaceutical carrier employed may be, for example, either a solid or liquid. Exemplary of solid carriers are lactose, terra alba, sucrose, talc, gelatin, agar, pectin, acacia, magnesium stearate, stearic acid and the like. Exemplary of liquid carriers are phosphate buffered saline solution, syrup, oil such as peanut oil and olive oil, water, emulsions, various types of wetting agents, sterile solutions and the like. Similarly, the carrier or diluent may include time delay material well known to the art, such as glyceryl monostearate or glyceryl distearate alone or with a wax.

~~The dosage regimen will be determined by the attending physician and other clinical~~ factors; preferably in accordance with any one of the above described methods. As is well known in the medical arts, dosages for any one patient depends upon many factors, including the patient's size, body surface area, age, the particular compound to be administered, sex, time and route of administration, general health, and other drugs being administered concurrently. Progress can be monitored by periodic assessment.

Furthermore, the use of pharmaceutical compositions which comprise antisense-oligonucleotides which specifically hybridize to RNA encoding mutated versions of a CYP3A4, CYP3A7 or hPXR gene according to the invention or which comprise antibodies specifically recognizing mutated CYP3A4, CYP3A7 or hPXR protein but not or not substantially the functional wild-type form is conceivable in cases in which the concentration of the mutated form in the cells should be reduced.

Thanks to the present invention the particular drug selection, dosage regimen and corresponding patients to be treated can be determined in accordance with the present invention. The dosing recommendations will be indicated in product labeling by allowing the prescriber to anticipate dose adjustments depending on the considered patient group, with information that avoids prescribing the wrong drug to the wrong patients at the wrong dose.

Furthermore, the present invention relates to a diagnostic composition or kit comprising any one of the afore-described polynucleotides, vectors, host cells, variant CYP3A4, CYP3A7 and hPXR proteins, antibodies, inhibitors, nucleic acid molecules or the corresponding vectors of the invention, and optionally suitable means for detection.

The kit of the invention may contain further ingredients such as selection markers and components for selective media suitable for the generation of transgenic cells and animals.

The kit of the invention may advantageously be used for carrying out a method of the invention and could be, inter alia, employed in a variety of applications, e.g., in the diagnostic field or as research tool. The parts of the kit of the invention can be packaged individually in vials or in combination in containers or multicontainer units. Manufacture of the kit follows preferably standard procedures which are known to the person skilled in the art. The kit or diagnostic compositions may be used for methods for detecting expression of a mutant form of CYP3A4, CYP3A7 or hPXR gene in accordance with any one of the above-described methods of the invention, employing, for example, immunoassay techniques such as radioimmunoassay or enzymeimmunoassay or preferably nucleic acid hybridization and/or amplification techniques such as those described herein before and in the examples.

Some genetic changes lead to altered protein conformational states. For example, some variant CYP3A4, CYP3A7 or hPXR proteins may possess a tertiary structure that renders

them far less capable of facilitating drug metabolism and transcription initiation, respectively. Restoring the normal or regulated conformation of mutated proteins is the most elegant and specific means to correct these molecular defects, although it is difficult. Pharmacological manipulations thus may aim at restoration of wild-type conformation of the protein. Thus, the polynucleotides and encoded proteins of the present invention may also be used to design and/or identify molecules which are capable of activating the wild-type function of a CYP3A4, CYP3A7 or hPXR gene or protein.

In another embodiment the present invention relates to the use of a drug or prodrug for the preparation of a pharmaceutical composition for the treatment or prevention of a disorder diagnosed by the method described hereinbefore.

Furthermore, the present invention relates to the use of an effective dose of a nucleic acid sequence encoding a functional and expressible wild type CYP3A4, CYP3A7 or hPXR protein for the preparation of a pharmaceutical composition for treating, preventing and/or delaying a disorder diagnosed by the method of the invention. A gene encoding a functional and expressible CYP3A4, CYP3A7 or hPXR protein can be introduced into the cells which in turn produce the protein of interest. Gene therapy, which is based on introducing therapeutic genes into cells by *ex-vivo* or *in-vivo* techniques is one of the most important applications of gene transfer. Suitable vectors and methods for *in-vitro* or *in-vivo* gene therapy are described in the literature and are known to the person skilled in the art; see, e.g., Giordano, Nature Medicine 2 (1996), 534-539; Schaper, Circ. Res. 79 (1996), 911-919; Anderson, Science 256 (1992), 808-813; Isner, Lancet 348 (1996), 370-374; Muhlhauser, Circ. Res. 77 (1995), 1077-1086; Wang, Nature Medicine 2 (1996), 714-716; WO94/29469; WO 97/00957 or Schaper, Current Opinion in Biotechnology 7 (1996), 635-640, and references cited therein. The gene may be designed for direct introduction or for introduction via liposomes, or viral vectors (e.g. adenoviral, retroviral) into the cell. Preferably, said cell is a germ line cell, embryonic cell, or egg cell or derived therefrom, most preferably said cell is a stem cell.

As is evident from the above, it is preferred that in the use of the invention the nucleic acid sequence is operatively linked to regulatory elements allowing for the expression and/or targeting of the CYP3A4, CYP3A7 or hPXR protein to specific cells. Suitable gene delivery systems that can be employed in accordance with the invention may include liposomes,

receptor-mediated delivery systems, naked DNA, and viral vectors such as herpes viruses, retroviruses, adenoviruses, and adeno-associated viruses, among others. Delivery of nucleic acids to a specific site in the body for gene therapy may also be accomplished using a biolistic delivery system, such as that described by Williams (Proc. Natl. Acad. Sci. USA 88 (1991), 2726-2729). Standard methods for transfecting cells with recombinant DNA are well known to those skilled in the art of molecular biology, see, e.g., WO 94/29469; see also supra. Gene therapy may be carried out by directly administering the recombinant DNA molecule or vector of the invention to a patient or by transfecting cells with the polynucleotide or vector of the invention ex vivo and infusing the transfected cells into the patient.

In a preferred embodiment of the uses and methods of the invention, said disorder is cancer.

These and other embodiments are disclosed or are obvious from and encompassed by the description and examples of the present invention. Further literature concerning any one of the methods, uses and compounds to be employed in accordance with the present invention may be retrieved from public libraries, using for example electronic devices. For example the public database "Medline" may be utilized which is available on Internet, e.g. under <http://www.ncbi.nlm.nih.gov/PubMed/medline.html>. Further databases and addresses, such as <http://www.ncbi.nlm.nih.gov/>, <http://www.infobiogen.fr/>, [http://www.fmi.ch/biology/research\\_tools.html](http://www.fmi.ch/biology/research_tools.html), <http://www.tigr.org/>, are known to the person skilled in the art and can also be obtained using, e.g., <http://www.lycos.com>. An overview of patent information in biotechnology and a survey of relevant sources of patent information useful for retrospective searching and for current awareness is given in Berks, TIBTECH 12 (1994), 352-364.

The pharmaceutical and diagnostic compositions, uses, methods of the invention can be used for the diagnosis and treatment of all kinds of diseases hitherto unknown as being related to or dependent on variant CYP3A4, CYP3A7 and hPXR genes. The compositions, methods and uses of the present invention may be desirably employed in humans, although animal treatment is also encompassed by the methods and uses described herein.

### Brief description of the figures

**Figure 1:** Differences in the genetic makeup influence the efficacy and safety of drug treatment.

**Figure 2:** A current model of the regulation of CYP3A4 by hPXR.

**Figure 3:** (A) Structure of the CYP3A4 gene as described in Ref. by Hashimoto, Eur Biochem 218 (1993) 585-95 and confirmed in this study. Coding regions are indicated as filled rectangles, the non-coding 5' untranslated region as a dashed rectangle. Arrowheads represent the positions of oligonucleotides used to screen the coding region of the gene (see Table 2 for the oligonucleotide sequences). (B) Determination of the exon flanking sequences. Double-headed arrows indicate genomic regions amplified by PCR. Single-headed arrows indicate sequences obtained by direct sequencing of BAC clones. The sequences of oligonucleotides shown in (B) are given in Table 1.

**Figure 4:** (A) Structure of the hPXR gene. Coding regions are indicated as filled rectangles, non-coding 5' and 3' untranslated regions as dashed rectangles. Arrowheads represent the positions of oligonucleotides used to screen the coding region of the gene (see Table 5 for the oligonucleotide sequences). (B) Elucidation of the genomic structure of hPXR. Double-headed arrows indicate genomic regions amplified by PCR. Oligonucleotides used to isolate an hPXR-containing BAC clone are shown in bold. Single-headed arrows indicate sequences obtained by direct sequencing of this clone. (C) Differential expression of hPXR transcripts in the liver (L) and small intestine (SI) as investigated by PCR amplification of tissue-derived cDNA pools. Exon 1b is expressed in both target tissues of the gene whereas exon 1a is expressed only in the liver. The sequences of oligonucleotides shown in (B) and (C) are given in Table 3.

**Figure 5:** Polymorphisms in the CYP3A4 and CYP3A7 genes. Numbering of polymorphic sites within the exons of CYP3A4 is based on the GenBank sequence M14096. Numbering of polymorphic sites within the exons of CYP3A7 is based on the GenBank sequence gi4503232.

**Figure 6:** Polymorphisms in the hPXR gene. Numbering of polymorphic sites within the exons of hPXR is based on the GenBank sequence AF084645.

**Figure 7:** An enzymatic test for the CYP3A7 exon 11 C1229G (Thr409Arg) polymorphism. (A) The mutation eliminates the unique AlwNI restriction site from the exon 11-containing fragment amplified with oligonucleotides 3A442F and 3A438R (arrowheads). (B) Genotyping of a wild-type (wt/wt) and a heterozygous (wt/C1229G) DNA sample by means of the AlwNI restriction digest.

**Figure 8:** Genomic sequences and polymorphisms in CYP3A4, CYP3A7 and hPXR genes. Primers used for the amplification and sequencing (Tables 1 and 3), as well as splice sites are underlined. Thick underlined are polymorphic sites and they are shown as the wild-type and variant base, separated by an arrow.

The invention will now be described by reference to the following examples which are merely illustrative and are not to be construed as a limitation of the scope of the present invention.

### **Examples**

Genomic samples, isolated by standard techniques from 22 human livers, were obtained from Prof. Urs Meyer, Biozentrum der Universität Basel. Liver samples were obtained under consideration of all legal, ethical and medical requirement of the Biozentrum der Universität Basel. Blood samples from 95 individuals were obtained and processed by ion exchange chromatography methods (Qiagen) to isolate DNA. Blood samples were

obtained under consideration of all legal, ethical and medical requirement of Parexel International (Berlin).

**Example 1: Genomic organization and oligonucleotides for the amplification of the coding regions of CYP3A4, CYP3A7 and hPXR**

The genomic structure of CYP3A4 has been described in an earlier work (Hashimoto, Eur. J. Biochem. 218 (1993), 585-95); however, the published exon flanking sequences were too short to design oligonucleotides for exon amplification. In accordance with the present invention these sequences have been elucidated first by sequencing of PCR (Polymerase Chain Reaction)-amplified fragments containing parts of two neighboring exons and the intercalated intron. The primers used for the amplification of these fragments were derived from the published cDNA sequence (Beaune, Proc. Natl. Acad. Sci. USA 83 (1986), 8064-8, GenBank accession number M14096) upon consideration of the exon/intron organization of the gene (Table 1, Fig. 3). For exons flanked by larger introns, CYP3A4-containing bacterial artificial chromosome (BAC) clones (Control numbers 22300 and 22301) purchased from Genome Systems (St. Louis, MO, USA) were directly sequenced. The clones had been isolated by PCR using the CYP3A4PF and CYP3A4PR oligonucleotides (Table 1), which were derived from the promoter region of the gene (GenBank accession number D11131, Hashimoto, supra). The sequences thus obtained were used to design oligonucleotides for the amplification of the gene exons (Fig. 1). Their sequences, the amplification conditions, and the sizes of the resulting DNA fragments are given in Table 2. Besides the exon sequences, fragments amplified contain also some flanking intronic sequences, including the splice sites. The oligonucleotide 3A425R was derived from the non-coding part of CYP3A4 cDNA (exon 13, Beaune, supra, GenBank accession number M14096). The sequences of oligonucleotides to determine the exon/intron boundary of Exon 11 of CYP3A7 (3A720R and 3A721F) are shown **in bold** in Table 1.

In accordance with the present invention a similar strategy was applied to elucidate the genomic organization and to determine exon-flanking sequences of the CYP3A4 and CYP3A7 regulator, hPXR (Fig. 4A, B, Table 3). The gene consists of 10 exons and spans at least 19 kb of genomic DNA. The sequences at exon-intron junctions as well as exon and intron sizes are given in Table 4. Exons 1b and 1a are utilized alternately in the

formation of hPXR transcripts. This is evidenced by the amplification of liver and intestine cDNA pools (Clontech) with primers derived from exons 1b, 1a, and 2 (Fig. 4C), followed by sequencing. In agreement with this observation, exon 1A lacks a 3' consensus splice site. Furthermore, exon 1B and 1A sequences correspond to the divergent 5' sequences of the two cDNA hPXR clones described by Bertilsson et al. (23) (GenBank accession numbers AF084645 and AF084644). The two transcripts are differentially expressed in the target tissues of hPXR. Exon 1b is expressed both in the liver and in the small intestine whereas exon 1a is expressed only in the latter tissue (Fig. 1B).

The sequences thus obtained were used to design oligonucleotides for amplification of the gene exons. Their composition and the sizes of the resulting DNA fragments are given in Table 5. Besides the exon sequences, fragments amplified contain also some flanking intronic sequences, including the splice sites, as well as some 5' and 3'-UTR (untranslated region) sequences of the gene.

#### **Example 2: Isolation of genomic DNA, amplification, purification and sequencing of CYP3A4, CYP3A7 and hPXR gene fragments**

Genomic DNA was isolated using standard techniques from 22 liver samples and 95 blood samples obtained from unrelated Caucasians. Conditions for the amplification of CYP3A4, CYP3A7 and hPXR gene fragments by PCR are given in Tables 2 and 5, respectively. The complete sequences of the amplicons are given in Figure 8. The quality of amplicons was routinely checked by agarose gel electrophoresis. The fragments were then processed through PCR purification columns (Qiagen) which remove all the components of the PCR that could otherwise interfere with the subsequent sequencing reaction.

The sequencing reaction was performed using the dye-terminator method and the samples were then resolved on polyacrylamide gels (Perkin-Elmer 377 and 3700 sequencing machines). Both strands were routinely sequenced to assure high accuracy of the results and the detection of heterozygotes. The sequences were visually inspected for their quality and then analyzed for the presence of polymorphisms using the PHRED/PHRAP/POLYPHRED/CONSED software package (University of Washington, Seattle, USA).



### Example 3: Polymorphisms in the CYP3A4 and CYP3A7 gene

In accordance with the present invention, 22 or more DNA samples were screened for mutations in exons 3, 4, 5, 7 and 9 of the CYP3A4 gene, and in exon 11 of the CYP3A7 gene. Three novel mutations were detected in the samples screened, two of them result in ~~non-conservative amino acid exchanges~~ (Fig. 5, Table 6, Table 8, Figure 8). The numbering of nucleotide polymorphisms within the CYP3A4 transcript, and of positions of the resulting amino acid exchanges, is based on GenBank sequence M14096. For CYP3A7 GenBank sequence gi 4503232 was used as reference.

- a G235A single nucleotide polymorphism (SNP) in exon 3 of CYP3A4 in 6 of the 234 chromosomes screened. The SNP results in the non-conservative amino acid exchange Gly→Asp at position 56 of the CYP3A4 protein.
- a G→T SNP in intron 7 of CYP3A4 in 4 out of 44 chromosomes screened. The SNP is localized at position 33 of the intron sequence (the G of the 5' consensus splice site GT is taken as position 1).
- a C1229G SNP in exon 11 of CYP3A7 in 17 out of 232 chromosomes screened. The SNP results in the non-conservative amino acid exchange Thr→Arg at position 409 of the CYP3A7 protein.

### Example 4: Polymorphisms in the hPXR gene

In accordance with the present invention 22 DNA samples were screened for mutations in exons 1b, 2, 5/6, 7, 8, 9 and 9a of the hPXR gene. Three polymorphisms were detected in the samples screened (Fig. 6, Table 7, Table 8, Figure 8). The numbering of nucleotide polymorphisms within the hPXR transcript, and of positions of the resulting amino acid exchanges is based on GenBank hPXR sequence AF084645.

- A C149A SNP in exon 1b in 27 out of 44 chromosomes screened. Exon 1b is untranslated, i.e. the SNP has no effect on the amino acid sequence of the protein.
- a CC975TT SNP in exon 5/6 in 2 out of 44 chromosomes screened. The SNP is silent, i.e. it has no effect on the amino acid sequence of the protein.
- A C→T SNP in intron 7 in 8 out of 44 chromosomes screened. The SNP is localized at position 184 of the intron sequence (the G of the 5' consensus splice site GT is taken as position 1).

### Example 5: An enzymatic test for the CYP3A7 exon 11 C1229G polymorphism

The C1229G polymorphism detected in exon 11 of CYP3A7 results in the loss of an AlwNI restriction site (Fig. 7A). In accordance with the present invention an AlwNI-based test was developed for the genotyping of the C1229G allele. An example is shown in Fig. 7B. A digest of the 404 bp genomic fragment amplified from a wild-type sample (wt/wt) with primers 3A742F and 3A738R (Table 2) generates two fragments of 316 bp and 88 bp, respectively. In a heterozygous sample (wt/C1229G), approximately half of the DNA remains undigested, due to the loss of the restriction site in the mutant allele.

Because population genetics enables a calculation of the expected frequency of homozygous vs. heterozygous alleles of a defined gene (Hardy-Weinberg formula,  $p^2 + 2pq + q^2 = 1$ ), it is also possible to confirm the predicted (with that formula) distribution of homozygous vs. heterozygous alleles and deviations with the experimental findings. This can serve as internal control and confirmation that a detected sequence deviation indeed represents a novel allele.

**Table 1. Oligonucleotides used to determine the exon/intron boundaries of the CYP3A4 gene and of the exon 11 of CYP3A7 (in bold).**

Name	Position	Sequence (5' - 3')
T7	BAC CS	TAATACGACTCACTATAGGG
3A43F	exon 2	GAACCCATTACATGGAC
3A46R	exon 4	TGATCATGTCAGGATCTG
3A47F	exon 4	GGTCAACAGCCTGTGCTG
3A48R	exon 5	TCCAGTGGTGAAGGTGG
3A49F	exon 5	GTGCCATCTCTATAGGTG
3A410R	exon 6	CTTCCCGCCTCAGATTTC
3A411F	exon 6	GAAATCTGAGGCGGGAAG
3A412R	exon 7	GGGTCTTGTGGATTGTTG
3A413F	exon 7	CAACAATCCACAAGACCC
3A414R	exon 8	GTGTATCTTCGAGGCGAC
3A415F	exon 8	CTTCCATTCTCATCCC

3A416R	exon 9	CCTTTGTGGGACTCAGTTTC
3A419F	exon 10	GCCACTCACCCTGATGTC
3A720R	exon 11	ATCACCACCCACCCTTG
3A721F	exon 11	CAAAGGGTGGGTGGTGAT
3A422R	exon 12	GAGAGCAAACCTCATGCC
3A423F	exon 12	GGCATGAGGTTTGCTCTC
3A424R	exon 13	GGTGCCATCCCTTGACTC
3A426R	exon 2	GCAGAGGTGTGGGCCCTG
3A4436F	intron 8	GGAGATCAAGGACCACGCTTG TG
3A441R	intron 10	CTTACGCTTCTGCCAGTAGCAACC
CYP3A4PF	promoter	AACAGGCGTGGAACACAAT
CYP3A4PR	promoter	CTTTCCTGCCCTGCACAG

Fifty ng of genomic DNA was added to a reaction mix (total volume 30 or 50 µl) containing 1x PCR buffer (Q=Qiagen, Cat.Nr. 1005479, or B2=Boehringer (currently Roche) Expand Long Template PCR Buffer number 2, Cat. Nr. 1742655), 0.25 µM each oligonucleotide (Metabion), 200 µM dNTPs, and 1 U of Taq polymerase (Qiagen). Amplifications were performed on a RoboCycler Gradient 96 (Stratagene) with an initial denaturation step of 2 min. at 94 °C followed by 32 amplification cycles of denaturation (40 sec., 94 °C), annealing (45 sec., temperatures 56-60 °C), and extension (60-150 sec., 72 °C). This was followed by a final extension step 5 min., 72 °C. The sequencing of PCR fragments and BAC clones was performed on a GeneAmp PCR System 9700 (Perkin-Elmer) using a dye-terminator DNA sequencing kit. (Perkin-Elmer, Cat.Nr. 4303154), according to manufacturers's instructions.

Table 2. CYP3A4 (exons 3, 4, 5, 7 and 9) and CYP3A7 (exon 11, in bold) polymorphism screen: oligonucleotide sequences, amplification conditions and fragment size.

Exon	Upstream Oligonucleotide		Downstream Oligonucleotide		Ann. Temp. (°C)	Product Size (bp)
	Name	Sequence (5'-3')	Name	Sequence (5'-3')		
1						
2						
3	3A450F	CCTCTAACTGCCAGCAAGTCTG	3A451R	GCGGTGAGACTGTCTCTGTG	58	247
4	3A452F	AGTCTGGCTTCCTGGGTTGGGCTC	3A437R	GAACTGGACGTGGAACCTTCCTGGAC	58	293
5	3A428F	CTACAACCATGGAGACCTCC	3A429R	TACCTGTCCCCCACCAGATTG	58	236
6						
7	3A433F	GTCGTCTTGACTGGACATGTGG	3A434R	GATGATGGTCACACATATCTTC	58	393
8						
9	3A436F	GGAGATCAAGGACCACGCTTGIG	3A447R	CTCATCATCCTGGAATACTTCCTGC	60	245
10						
11	3A742F	CCAGTATGAGTTGTTCTCTGG	3A738R	AGGCAGAATATGCTTGAACCCAGGC	58	404
12						
13						

Fifty ng of genomic DNA was added to a reaction mix containing 1x PCR buffer (Qiagen), 0.5  $\mu$ M oligonucleotides, 200  $\mu$ M dNTPs, and 1 U of Taq polymerase (Qiagen). Amplifications were performed on a RoboCycler Gradient 96 (Stratagene) with an initial denaturation step of 2 min. at 94 °C followed by 32 amplification cycles of denaturation (40 sec., 94 °C), annealing (45 sec., temperatures given above), and extension (60 sec., 72 °C). This was followed by a final extension step 5 min., 72 °C. All sequencing reactions were performed on a GeneAmp PCR System 9700 (Perkin-Elmer) using a dye-terminator DNA sequencing kit (Perkin-Elmer, catalog number 4303154), according to manufacturers's instructions.

**Table 3.** Oligonucleotides used to determine the structure and exon/intron boundaries of the hPXR gene.

Name	Position	Sequence (5' - 3')
hPXR1F	exon4	TCATGTCCGACGAGGCCG
hPXR4F	exon 5/6	CCCACATGGCTGACATGT
hPXR5F	exon 7	CCCATCGAGGACCAGATC
hPXR6R	exon 7	GTCTTCCAAGCAGTAGGA
hPXR7R	exon 8	CAGCATGGGCTCCAGTAG
hPXR10R	exon 9a	CCTGTGATGCCGAACAAC
hPXR11F	exon 9	CATTGAATGCAATCGGCC
hPXR12R	exon 9a	GCTCTTGGCAGTGTCCAT
hPXR15F	exon 2	GGAAAGCCCAGTGTCAAC
hPXR16F	exon 3	CCATGAAACGCAACGCCC
hPXR18R	exon 2	CCTTGCATCCTTCACATG
hPXR19R	exon 3	CATGCCGCTCTCCAGGCA
hPXR20R	exon 4	CGGCCTCGTCGGACATGA
hPXR21R	exon 5/6	ACATGTCAGCCATGTGGG
hPXR47F	exon 1b	CAAGCCAAGTGTTCACAGTG
hPXR48R	exon 1b	CACTGTGAACACTTGGCTTG
hPXR52F	exon 1a	CAAGGACAGCAGCATGACAGTCAC
hPXR54R	exon 1a	AGCCAACTCAGCCGCAGC

Fifty ng of genomic DNA was added to a reaction mix (total volume 30 or 50 µl) containing 1x PCR buffer (Q=Qiagen, Cat.Nr. 1005479, or B2=Boehringer (currently Roche) Expand Long Template PCR Buffer number 2, Cat. Nr. 1742655); 0.25 µM each oligonucleotide (Metabion); 200 µM dNTPs, and 1 U of Taq polymerase (Qiagen). Amplifications were performed on a RoboCycler Gradient 96 (Stratagene) with an initial denaturation step of 2 min. at 94 °C followed by 32 amplification cycles of denaturation (40 sec., 94 °C), annealing (45 sec., temperatures 56-60 °C), and extension (60-150 sec., 72 °C). This was followed by a final extension step 5 min., 72 °C All sequencing reactions were performed on a GeneAmp PCR System 9700 (Perkin-Elmer) using a dye-terminator DNA sequencing kit (Perkin-Elmer, Cat.Nr. 4303154), according to manufacturers's instructions

**Table 4.** Exon-intron organization of the hPXR gene.

Exon number	Exon size (b)	Sequence at exon-intron junction		Intron size (kb)
		5' splice site	3' splice site	
1b	281	AAGCAG gtagg....	....tcttag TCCAAG	0.403
1a	144	AAAGCA gtagg....	....tcttag TCCAAG	7.0
2	219	TTTCAG gtagg....	....tcacag GAGGGC	12.7
3	134	AGGAGA gtagg....	....ctgcag TGAACA	1.2
4	188	TTCCGG gtagg....	....tcttag CTGCCA	~ 1.0
5/6	275	CTTCAG gtagg....	....tgccag GGACTT	~ 1.9
7	143	CTGCAG gtagg....	....ccacag GTGGCT	0.201
8	117	CCCCAG gtagg....	....ctccag ACCGCC	0.286
9	106	TCATAG gtagg....	....atgcag GTTCTT	~ 1.3
9a	1418			

The sizes of exons 1b and 1a based on a comparison of genomic (Figure 8), and cDNA (GenBank accession numbers AF061056, AF084645, AF084644) hPXR sequences.

Table 5. hPXR polymorphism screen: oligonucleotide sequences, amplification conditions and fragment size.

Exon	Upstream Oligonucleotide		Downstream Oligonucleotide		Ann. Temp. (°C)	Buffer	Product Size (bp)
	Name	Sequence (5'-3')	Name	Sequence (5'-3')			
1b	HPXR57F	TCAAGTGTGGACTTGGGAC	HPXR58R	CCCACATGATGCTGACCTC	53	B2	460
1a							
2	HPXR41F	CTGAGGCCTCTACACATC	HPXR40R	AGGCCCTGAGATGTTACC	55	Q	345
3							
4							
5/6	HPXR32F	CTGAGTTGGGACCTGTCT	HPXR35R	CCAGGCCCTTTGAACCTC	60	B2	415
7&8	HPXR36F	CTGCTGGTGCCTGGCCTGT	HPXR33R	GACTGGGACCTTCCCTGG	60	B2	598
9	HPXR34F	GAGCAATGCCCTGACTCT	HPXR26R	CCCTCTGGCCATGAAGTC	60	B2	271
9a	HPXR30F	TGCTTGTCAGCCTCAGA	HPXR12R	GCTCTTGGCAGTGTCAT	60	B2	324

Preliminary exon numbering. Fifty ng of genomic DNA was added to a reaction mix (total volume 30 or 50 µl) containing 1x PCR buffer (Q=Qiagen, Cat.Nr. 1005479, or B2=Boehringer (currently Roche) Expand Long Template PCR Buffer number 2, Cat. Nr. 1742655), 0.25 µM each oligonucleotide (Metabion), 200 µM dNTPs, and 1 U of Taq polymerase (Qiagen). Amplifications were performed on a RoboCycler Gradient 96 (Stratagene) with an initial denaturation step of 2 min. at 94 °C followed by 32 amplification cycles of denaturation (40 sec., 94 °C), annealing (45 sec., temperatures given above), and extension (60 sec., 72 °C). This was followed by a final extension step 5 min., 72 °C All sequencing reactions were performed on a GeneAmp PCR System 9700 (Perkin-Elmer) using a dye-terminator DNA sequencing kit (Perkin-Elmer, Cat.Nr. 4303154), according to manufacturers's instructions.

**Table 6.** Distribution of polymorphisms in CYP3A4 (exons 3, 4, 5, 7 and 9) and CYP3A7 (exon 11, in bold) in a set of 22 DNA samples.

Number	Donor Number	Exons												
		1	2	3	4	5	6	7	8	9	10	11	12	13
1	11			-	-	-	-	-	-	-	-	-	-	-
2	17			-	-	-	-	-	-	-	-	-	-	-
3	18			-	-	-	-	-	-	-	-	X	-	-
4	19			-	-	-	-	-	-	-	-	-	-	-
5	20			-	-	-	-	-	-	-	-	-	-	-
6	21			-	-	-	-	-	-	-	-	-	-	-
7	22			-	-	-	-	-	-	-	-	-	-	-
8	23			-	-	-	-	-	-	-	-	-	-	-
9	24			-	-	-	-	X	-	-	-	X	-	-
10	25			-	-	-	-	-	-	-	-	X	-	-
11	27			-	-	-	-	-	-	-	-	-	-	-
12	29			-	-	-	-	-	-	-	-	-	-	-
13	31			-	-	-	-	-	-	-	-	-	-	-
14	33			-	-	-	-	-	-	-	-	-	-	-
15	34			-	-	-	-	-	-	-	-	-	-	-
16	35			-	-	-	-	-	-	-	-	X	-	-
17	36			-	-	-	-	-	-	-	-	-	-	-
18	37			-	-	-	-	-	-	-	-	-	-	-
19	38			-	-	-	-	X	-	-	-	-	-	-
20	40			X	-	-	-	-	-	-	-	-	-	-
21	41			-	-	-	-	X	-	-	-	X	-	-
22	43			-	-	-	-	X	-	-	-	X	-	-

"-"=wild-type, "X"=heterozygote, "XX"=homozygote



**Table 7.** Distribution of hPXR gene polymorphisms in a set of 22 DNA samples.

Number	Donor	Exons									
	Number	1b	1a	2	3	4	5/6	7	8	9	9a
1	11			-			-	X	-	-	-
2	17	X		-			-	-	-	-	-
3	18	XX		-			-	-	-	-	-
4	19	X		-			-	-	-	-	-
5	20	X		-			-	-	-	-	-
6	21	XX		-			-	X	-	-	-
7	22	XX		-			-	-	-	-	-
8	23	XX		-			-	-	-	-	-
9	24	XX		-			-	-	-	-	-
10	25	X		-			-	-	-	-	-
11	27	-		-			XX	XX	-	-	-
12	29	XX		-			-	X	-	-	-
13	31	X		-			-	X	-	-	-
14	33	-		-			-	-	-	-	-
15	34	X		-			-	-	-	-	-
16	35	XX		-			-	-	-	-	-
17	36	X		-			-	-	-	-	-
18	37	X		-			-	-	-	-	-
19	38	-		-			-	X	-	-	-
20	40	XX		-			-	X	-	-	-
21	41	X		-			-	-	-	-	-
22	43	XX		-			-	-	-	-	-

"-"=wild-type. "X"=heterozygote. "XX"=homozygote

Table 8. Genetic variants of CYP3A4, CYP3A7 and hPXR.

Gene	Position	wt sequence (5'-3')	mut sequence (5'-3')
CYP3A4	exon 3	F: TCCCAGGGCTTTTGT R: <del>ACAAAAGCCCTGCCA</del>	F: TCCCAGGACTTTTGT R: <del>ACAAAAGTCCCTGCCA</del>
	intron 7	F: <del>TATCTTCTCTGTT</del> R: <del>AAGAGAGAAAGATA</del>	F: <del>TATCTTGCTCTCTT</del> R: <del>AAGAGAGCAAGATA</del>
CYP3A7	exon 11	F: TACTGGACAGAGC R: GCTCTGTCCAGTA	F: TACTGGAGAGAGC R: GCTCTCTCCAGTA
hPXR	exon 1b	F: CCTGAACAAGGCAG R: CTGCCTTGTTTCAGG	F: CCTGAAAAGGCAG R: CTGCCTTTTTCAGG
	exon 5/6	F: <del>CAGTGGCGCGAAA</del> R: <del>TTTCCCGCCACTG</del>	F: <del>CAGTGGCTCGCGAAA</del> R: <del>TTTCCCGCCACTG</del>
	intron 7	F: <del>CCTCCATCCTGTTAC</del> R: <del>GTAAACAGCATCGAGC</del>	F: <del>CCTCCATTCTGTTAC</del> R: <del>GTAAACAGCATCGAGC</del>

10. Sep. 1999

## References

- (1) Daly, Toxicol Lett 102-103 (1998), 143-7
  - (2) Touw, Drug Metabol Drug Interact 14 (1997), 55-82
  - (3) Thummel, Annu Rev Pharmacol Toxicol 38 (1998), 389-430
  - (4) Cholerton, Trends Pharmacol Sci 13 (1992), 434-9
  - (5) Ketter, J Clin Psychopharmacol 15 (1995), 387-98

---

  - (6) Forrester, Proc Natl Acad Sci U S A 87 (1990), 8306-10
  - (7) Paolini, Nature 398 (1999), 760-1
  - (8) Westlind, Biochem Biophys Res Commun 259 (1999), 201-5
  - (9) Jounaidi, Biochem Biophys Res Commun 221 (1996), 466-70
  - (10) Schuetz, Pharmacogenetics 4 (1994), 11-20
  - (11) Hunt, Clin Pharmacol Ther 51 (1992), 18-23
  - (12) Kashuba, Clin Pharmacol Ther 64 (1998), 269-77
  - (13) Peyronneau, Eur J Biochem 218 (1993), 355-61
  - (14) Rebbeck, J Natl Cancer Inst 90 (1998), 1225-9
  - (15) Felix, Proc Natl Acad Sci U S A 95 (1998), 13176-81
  - (16) He, Biochemistry 36 (1997), 8831-9
  - (17) Szklarz, J Comput Aided Mol Des 11 (1997), 265-72
  - (18) Harlow, J Biol Chem 272 (1997), 5396-402
  - (19) Wang, Biochemistry 37 (1998), 12536-45
  - (20) Harlow, Proc Natl Acad Sci U S A 95 (1998), 6636-41
  - (21) Domanski, Arch Biochem Biophys 350 (1998), 223-32
  - (22) Lehmann, J Clin Invest 102 (1998), 1016-23
  - (23) Bertilsson, Proc Natl Acad Sci U S A 95 (1998), 12208-13
  - (24) Kliever, Cell 92 (1998), 73-82
  - (25) Pascussi, Biochem Biophys Res Commun 260 (1999), 377-81
-



.

.



## Claims

EPO-Munich  
60

10. Sep. 1999

1. A polynucleotide selected from the group consisting of:
  - (a) polynucleotides comprising a nucleotide sequence encoding the amino acid sequence encodable by a nucleotide sequence of SEQ ID NO: 86, 94 and/or 98;
  - (b) ~~polynucleotides comprising a nucleotide sequence encoding a molecular~~ variant of the cytochrome CYP3A4 protein by way of at least one amino acid deletion, addition and/or substitution at an amino acid position corresponding amino acid residue Gly56 in exon 3 of the CYP3A4 gene;
  - (c) polynucleotides comprising a nucleotide sequence encoding a molecular variant of the cytochrome CYP3A7 protein by way of at least one amino acid deletion, addition and/or substitution, preferably at an amino acid position corresponding amino acid residue Thr 409 in exon 11 of the CYP3A7 gene;
  - (d) polynucleotides comprising a nucleotide sequence of any one of SEQ ID NOS: 86, 87, 90, 91, 94, 95 or 108;
  - (e) polynucleotides comprising a nucleotide sequence of any one of SEQ ID NOS: 98, 99, 102, 103, 106 or 107; and
  - (f) polynucleotides comprising a nucleotide sequence encoding a CYP3A4 or CYP3A7 polypeptide or fragment thereof having an epitope comprising an amino acid sequence encoded by the nucleotide sequence of SEQ ID NO: 86 and/or 94.
2. The polynucleotide of claim 1, wherein said polynucleotide encodes a variant CYP3A4, CYP3A7 or hPXR protein or fragment thereof.
3. The polynucleotide of claim 1 or 2, wherein said amino acid substitution comprises Gly56 to Asp in exon 3 of the CYP3A4 gene and/or Thr409 to Arg in exon 11 of the CYP3A7 gene.
4. The polynucleotide of any one of claims 1 to 3, wherein the nucleotide deletion, addition and/or substitution result in altered expression of the variant CYP3A4, CYP3A7 or hPXR gene compared to the corresponding wild type gene.

5. A vector comprising the polynucleotide of any one of claims 1 to 4.
6. The vector of claim 5, wherein the polynucleotide is operatively linked to expression control sequences allowing expression in prokaryotic or eukaryotic cells.
7. A host cell genetically engineered with the polynucleotide of any one of claims 1 to 4 or the vector of claim 5 or 6.
8. A method for producing a molecular variant CYP3A4, CYP3A7 or hPXR protein or fragment thereof comprising
  - (a) culturing the host cell of claim 7; and
  - (b) recovering said protein or fragment from the culture.
9. A method for producing cells capable of expressing a molecular variant CYP3A4, CYP3A7 or hPXR gene comprising genetically engineering cells with the polynucleotide of any one of claims 1 to 4 or the vector of claim 5 or 6.
10. A CYP3A4, CYP3A7 or hPXR protein or fragment thereof encoded by the polynucleotide of any one of claims 1 to 4 or obtainable by the method of claim 8 or from cells produced by the method of claim 9.
11. An antibody which binds specifically to the protein of claim 10.
12. The antibody of claim 11 which specifically recognizes an epitope containing one or more amino acid substitution(s) as defined in any one of claims 1 to 4.
13. A nucleic acid molecule complementary to a polynucleotide of any one of claims 1 to 4.
14. A nucleic acid molecule capable of specifically recognizing and cleaving the polynucleotide of any one of claims 1 to 4.
15. A vector comprising the nucleic acid molecule of claim 13 or 14.

16. A transgenic non-human animal comprising at least one polynucleotide of any one of claims 1 to 4 or the vector of claim 5 or 6.
17. The transgenic non-human animal of claim 16 further comprising at least one inactivated wild type allele of the CYP3A4, CYP3A7 or hPXR gene.
18. The transgenic non-human animal of claim 16 or 17, which is a mouse or a rat.
19. A method of identifying and obtaining a CYP3A4, CYP3A7 or hPXR inhibitor capable of modulating the activity of a molecular variant of the CYP3A4, CYP3A7 or hPXR gene or its gene product comprising the steps of
  - (a) contacting the protein of claim 10 or a cell expressing a molecular variant CYP3A4, CYP3A7 or hPXR gene comprising a polynucleotide of any one of claims 1 to 4 in the presence of components capable of providing a detectable signal in response to drug metabolism, with a compound to be screened under conditions to permit CYP3A4- or CYP3A7-mediated drug metabolism, and
  - (b) detecting the presence or absence of a signal or increase of a signal generated from the drug metabolism, wherein the presence or increase of the signal is indicative for a putative inhibitor.
20. The method of claim 19 wherein said cell is a cell of claim 7, obtained by the method of claim 9 or is comprised in the transgenic non-human animal of any one of claims 16 to 18.
21. A method of identifying and obtaining an CYP3A4, CYP3A7 or hPXR inhibitor capable of modulating the activity of a molecular variant of the CYP3A4, CYP3A7 or hPXR gene or its gene product comprising the steps of
  - (a) contacting the protein of claim 10 with a first molecule known to be bound by CYP3A4, CYP3A7 or hPXR protein to form a first complex of said protein and said first molecule;
  - (b) contacting said first complex with a compound to be screened; and
  - (c) measuring whether said compound displaces said first molecule from said first complex.

22. The method of claim 21, wherein said measuring step comprises measuring the formation of a second complex of said protein and said compound.
23. The method of claim 21 or 22, wherein said measuring step comprises measuring the amount of said first molecule that is not bound to said protein.
24. The method of any one of claim 21 to 23, wherein said first molecule is nifedipine, rifampicine or corticosterone.
25. The method of any one of claims 21 to 24 wherein said first molecule is labeled.
26. A method of diagnosing a disorder related to the presence of a molecular variant of the CYP3A4, CYP3A7 or hPXR gene or susceptibility to such a disorder comprising:
- (a) determining the presence of a polynucleotide of any one of claim 1 to 4 in a sample from a subject; and/or
  - (b) determining the presence of a protein of claim 10.
27. The method of claim 26, wherein said disorder is cancer.
28. The method of claim 26 or 27 comprising PCR, ligase chain reaction, restriction digestion, direct sequencing, nucleic acid amplification techniques, hybridization techniques or immunoassays.
29. The method of any one of claims 26 to 28, further comprising administering to a subject a medicament to abolish or alleviate said disorder.
30. The method of any one of claims 26 to 29, further comprising introducing
- (i) a functional and expressible wild-type CYP3A4, CYP3A7 or hPXR gene
- or
- (ii) a nucleotide acid molecule of claim 13 or 14 or the vector of claim 15 into cells.



31. A method for the production of a pharmaceutical composition comprising the steps of the method of any one of claims 19 to 25; and  
(c) synthesizing and/or formulating the compound identified and obtained in step (b) or a derivative thereof in a pharmaceutically acceptable form.
32. A method for the preparation of a pharmaceutical composition comprising ~~formulating a drug or pro-drug in the form suitable for therapeutic application~~ and preventing or ameliorating the disorder of the subject diagnosed in the method of claim 26 or 27.
33. The method of claim 31 or 32 wherein said compound drug or prodrug is a derivative of a medicament as defined in claim 29.
34. An inhibitor identified or obtainable by the method of any one of claims 19 to 25.
35. The inhibitor of claim 34 which binds specifically to the protein of claim 10.
36. Use of an oligo- or polynucleotide for the detection of a polynucleotide of any one of claims 1 to 4 and/or for genotyping of individual CYP3A4, CYP3A7 or hPXR alleles.
37. The use of claim 36 wherein said polynucleotide is a polynucleotide of any one of claims 1 to 4 or a nucleic acid molecule of claim 13 or 14.
38. The use of claim 36 wherein said oligonucleotide is about 15 to 50 nucleotides in length and comprises the nucleotide sequence of any one of SEQ ID NOS: 1 to 107 or a complementary sequence.
39. A primer or probe consisting of an oligonucleotide as defined in claim 38.
40. Use of an antibody or a substance capable of binding specifically to the gene product of an CYP3A4, CYP3A7 or hPXR gene for the detection of the protein of claim 10, the expression of a molecular variant CYP3A4, CYP3A7 or hPXR gene comprising a polynucleotide of any one of claims 1 to 4 and/or for

distinguishing CYP3A4 or hPXR alleles comprising a polynucleotide of any one of claims 1 to 4.

41. A composition comprising the polynucleotide of any one of claims 1 to 4, the vector of claim 5 or 6, the host cell of claim 7 or obtained by the method of claim 9, the protein of claim 10, the antibody of claim 11 or 12, the nucleic acid molecule of claim 13 or 14, the vector of claim 15, the inhibitor of claim 34 or the primer or probe of claim 39.
42. The composition of claim 41 which is a diagnostic or a pharmaceutical composition.
43. Use of an effective dose of a drug or prodrug for the preparation of a pharmaceutical composition for the treatment or prevention of a disorder of a subject comprising a polynucleotide of any one of claims 1 to 4 in its genome.
44. The use of 43 wherein said disorder is cancer.

1/12

EPO-Munich  
60  
10. Sep. 1999

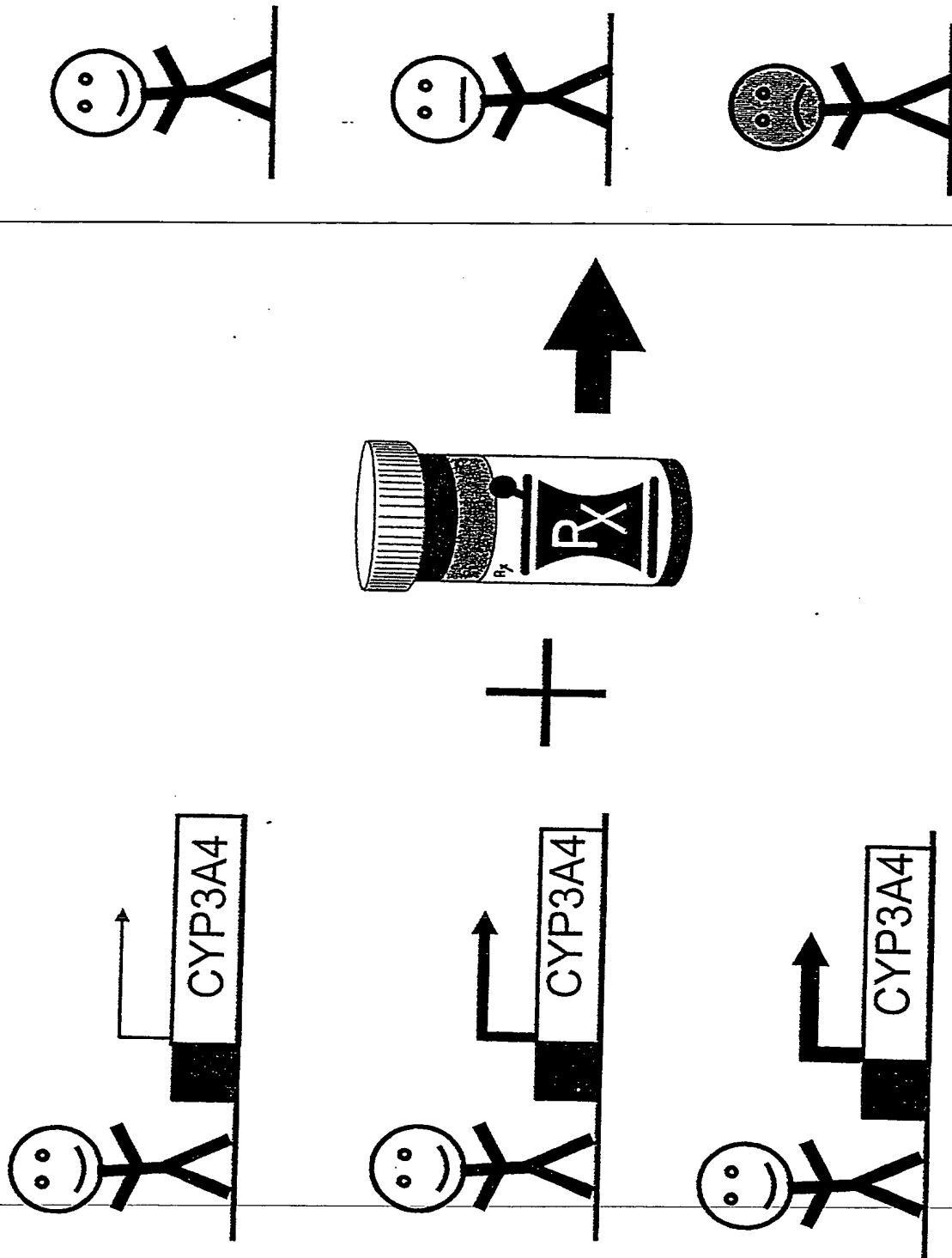
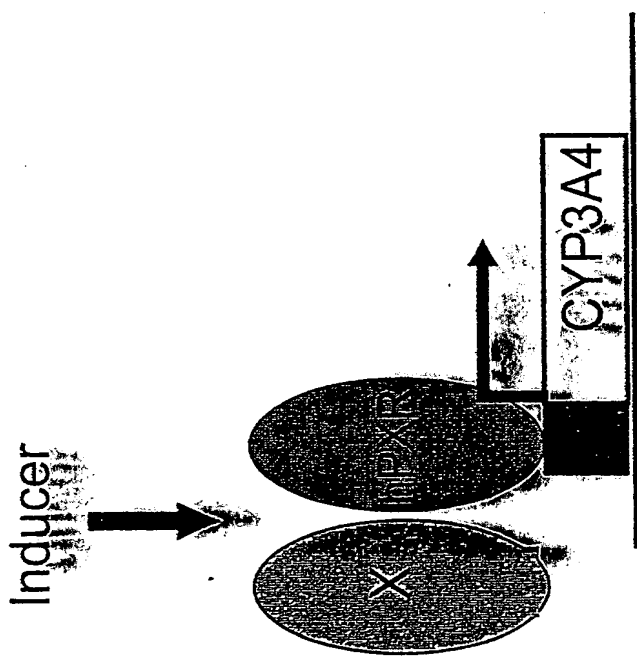


Fig. 1

Fig. 2



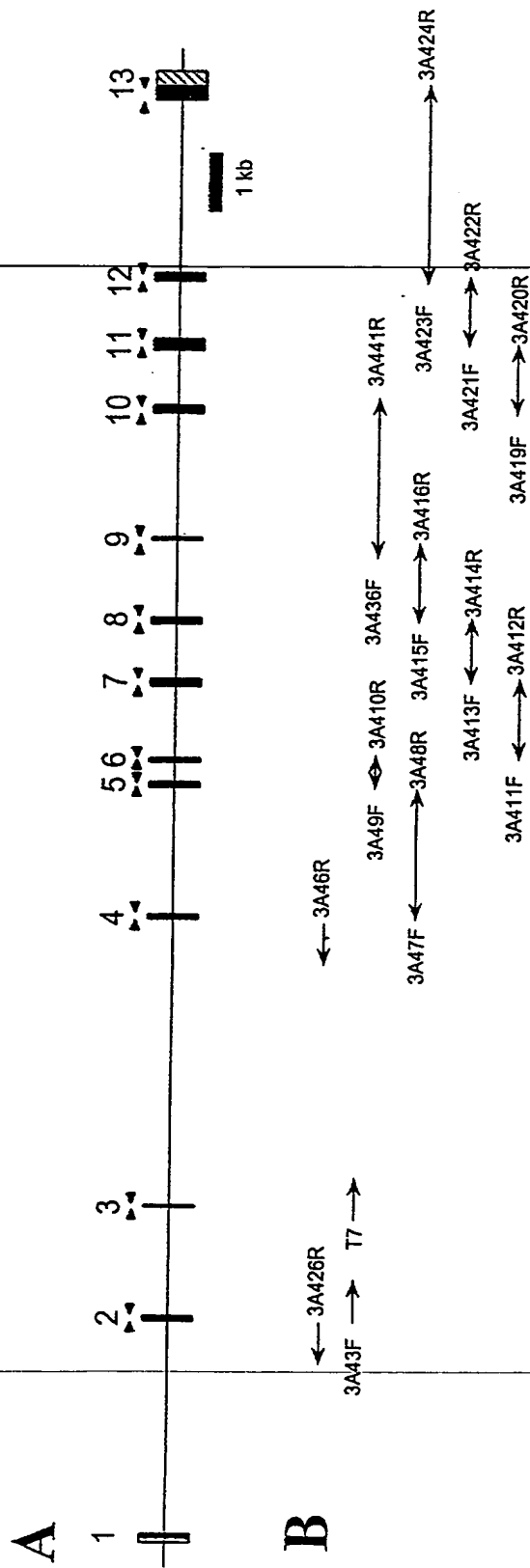


Fig. 3

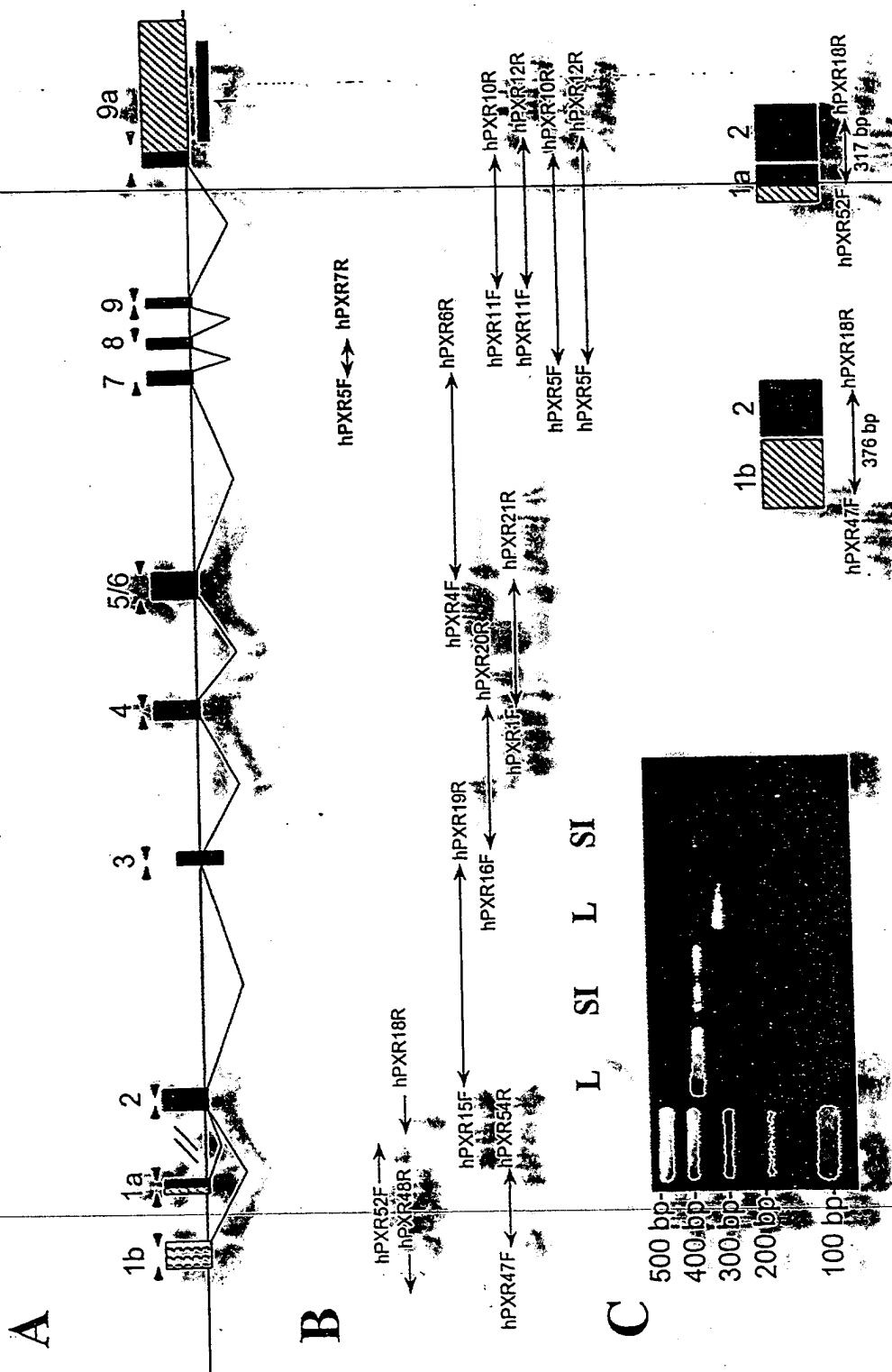


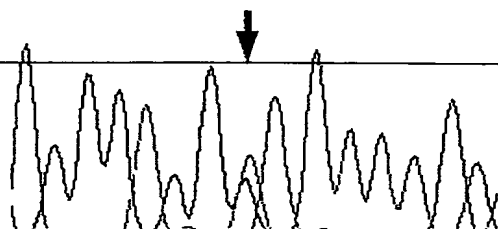
Fig. 4

5/12

## CYP3A4 polymorphisms

Exon 3 (G235A; Gly56Asp)

T C C C A G G N C T T T T G T

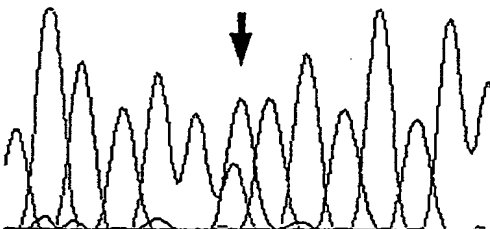


wt/mut

Oligonucleotide 3A450F (forwards)

Intron 7 (T→G)

T A T C T T G C T C T C T T

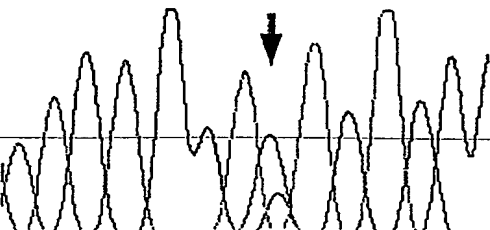


wt/mut

Oligonucleotide 3A433F (forwards)

A CYP3A7 exon 11 polymorphism (C1229G; Thr409Arg)

T A C T G G A C A G A G C C



wt/mut

Oligonucleotide 3A742F (forwards)

Fig. 5

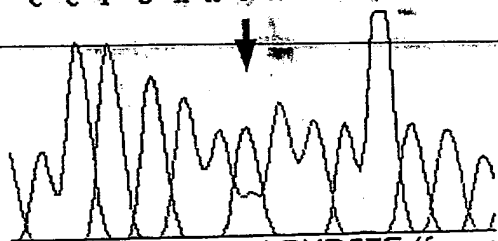
6/12

## hPXR polymorphisms

Exon 1b (C149A, untranslated)

wt/mut

C C T G A A C A A G A G C A A G



Oligonucleotide hPXR57F (forwards)

Exon 5/6 (CC975T, silent)

wt/wt

C C A G A T G G C C G G G A A A A A

Oligonucleotide hPXR32F (forwards)  
(forwards)

mut/mut

C C A G A T G G C C G G G A A A A A



Oligonucleotide hPXR32F

Intron 7 (C→T)

wt/mut

C C T C C A T T C T G T T A C



Oligonucleotide hPXR36F (forwards)

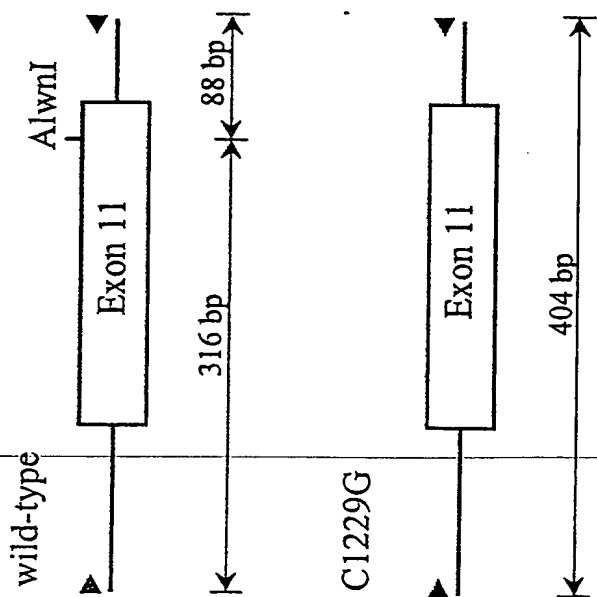
Fig. 6



7/12

Fig. 7

A



B

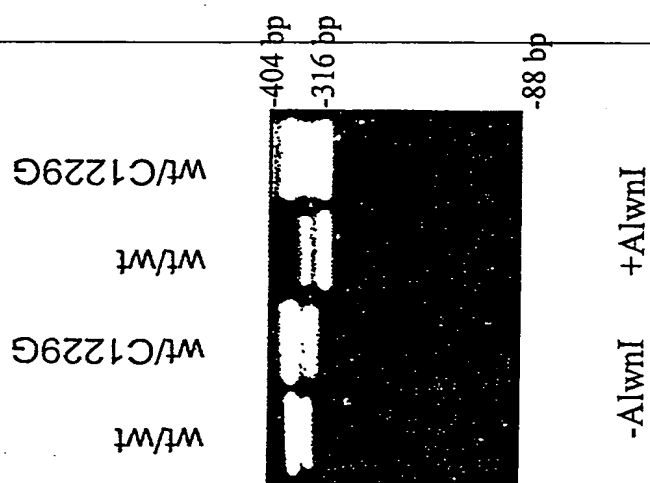


Fig. 8

CYP3A4

Exon 3 (G235A Gly56Asp)

CCTCTAACTGCCAGCAAGTCTGATTTTCATTGGCTTCGACTGTTTTTCATCCCAATTAGAGG  
CAGGGTTAAGTACATTAAAAATAATAATCAAATATTATTTGTTTCTCCTCCCAGGG→AC  
TTTTGTATGTTTGACATGGAATGTCATAAAAAGTATGGAAAAGTGTGGGGGTGAGTATT  
CTGGAAACTTCCATTGGATAGACTTGTTTCTATGATGAGTTTACCCCACTGCACAGAGGA  
CAGTCTCAGCCC

## Exon 4

AGTCTGGCTTCCTGGGTTGGGCTCCAGCTGTAGAATAAGGCTGTTGATGTTTAATCAAC  
TCTGTTTTTTTACACACAGCTTTTATGATGGTCAACAGCCTGTGCTGGCTATCACAGATC  
CTGACATGATCAAAACAGTGCTAGTGAAAGAATGTTATTCTGTCTTCACAAACCGGAG  
GGTAAGCATTCATGTGTTGAAATTAATACTGATTGATTAAATTTATATTTTGAATTCTT  
ATATATTCATAGACAGTTGCCTAAAAAATGTCCAGGAAGGTTCCACGTTCCACTTC

## Exon 5

CTACAACCATGGAGACCTCCACAACCTGATGTAGGACAAAATGTTTCTGCTTTGAACTCT  
AGCCTTTTGGTCCAGTGGGATTTATGAAAAGTGCCATCTCTATAGCTGAGGATGAAGA  
ATGGAAGAGATTAGGATCATTTGGTGTCTCCAAACCTTCACCAAGTGGAAAACCTCAAGGA  
GGTATGAAAATAACATGAGTTTTTAATAAGAACTTAAAGAATGAATCTGGTGGGGACAG  
GTA

## Exon 7 (T/G intron 7 polymorphism)

GTCTGTCTTGACTGGACATGTGGCTTTCTGATGCACGCATAGAGGAAGGATGGTAAA  
AAGGTGCTGATTTTAATTTCCACATCTTTCTCCACTCAGCGTCTTTGGGGCCTACAGC  
ATGGATGTGATCACTAGCACATCATTGGAGTGAACATCGACTCTCTCAACAATCCAC  
AAGACCCCTTTGTGGAAAACACCAAGAAGCTTTTAAGATTGATTTTTTGATCCATTCT  
TTTCTCTCAATAAGTATGTGGACTAGTATTTCTTTTATTTATCTTT→GCTCTCTTAAAAA  
TAACTGCTTTATTGAGATATAAATCAGCATGTAAATTCATCCAGTTAAATATACAGTTCAG  
TGATTTGTAGTACATTTGAAGATAATGCTGAGCATCATC

9/12

## Fig. 8 (continued)

## Exon 9

GGAGATCAAGGACCACGCTTGTGATTTACTTCTGACTTCAGGAGCCACTTTCTGTCAGT  
GAAATTTCTCTTTTGTCTTAGCACCGAGTGGATTTCTTCAGCTGATGATTGACTCTC  
AGAATTCAAAGAACTGAGTCCACAAAGGTAACCAGAGTGTTTCTGAGGGCTACTT  
GTGGGGCACTCAGAGGGAAGGCCTTGTTCTGAAAATGTGCAGGAAGTATTCCAGGATG  
ATGAG

CYP3A7

## Exon 11 (C1229G Thr409Arg polymorphism)

CCAGTATGAGTTGTTCTCTGGAACTTCTAACAGTTCAACAGTACTACATGGACTGAGTTA  
AAAGTTAATTCAAAAATCTCAATTTATCCAAATCTGTTTCTTTCTTTTCAGGCACCACCCA  
CCTATGATACTGTGCTACAGTTGGAGTATCTTGACATGGTGGTGAATGAAACACTCAG  
ATTATTCCCAGTTGCTATGAGACTTGAGAGGGTCTGCAAAAAAGATGTTGAAATCAAT  
GGGATGTTTATTCCCAAAGGGGTGGTGGTGATGATTCCAAGCTATGTTCTTCATCATG  
ACCCAAAGTACTGGAC→GAGAGCCTGAGAAGTTCCTCCCTGAAAGGTAGGAGGCC  
CTGGGAAGGGAGCCCTCCCTGAACCAGCCTGGTTCAAGCATATTCTGCCT

hPXR

Exon 1b&1a (in bold in exon 1b: nn 1-280 of hPXR cDNA (GenBank AF061056.1),  
dotted: nn 36-257 of PAR1 cDNA (GenBank AF084645))

TCAAGTGCTGGACTTGGGACTTAGGAGGGGCAATGGAGCCGCTTAGTGCCTACATCTG  
ACTTGGACTGAAATATAGGTGAGAGACAAGATTGTCTCATATCCGGGGAAATCATAAC  
CTATGACTAGGACGGGAAGAGGAAGCACTGCCTTTACTTCAGTGGGAATCTCGGCCT  
CAGCCTGCAAGCCAAGTGTTACAGTGAAAAAGCAAGAGAATAAGCTAATACTCCT  
GTCCTGAAC→AAAGGCAGCGGCTCCTTGGTAAAGCTACTCCTTGATCGATCCTTTGCA  
CCGGATTGTTCAAAGTGGACCCAGGGGAGAAGTCGGAGCAAAGAACTTACCACCAA  
GCAGGATGGTTTTCTTTCTTTCTTTTCTGGGGGCTGACCGCCCTTCAGCTCCAG  
CCAAAAGATGTGTGTGAACACAAATATACCTTCTGTT

10/12

Fig. 8 (continued)

TGAGGTCAGCATCATAGTGGGTCGTGAATCATGTTGGCCTTGCTGCTGTCTCCTCATTT  
CTAGGGTGAAAAAAGCATGAAAACAATCACTTAATGTTGAGCCCCATTA  
CTGATGCTCTCTGGTCTCTGCACTAGGCTCCTAGAAAAATCACCAGAGCCTTAACACTG  
CATGAGTTACCACAAGTCACACATACAACCAGCTCCCTGTTACAGGGGCTGGAGTCCCTG  
GACCCAGGAAATACCACCTCCAAGGACTGTGGGAGCTGGGGACTATGGGAAGTGGGAT  
CAACTCAGTCCTGATTCTTTTGGCCTGCTGGGTAGTGCTGGCAGCCCCCTGAGGC  
CAAGGACAGCAGCATGACAGTCACCAGGACTCACCACCTTCAAGGAGGGGTCCCTCAG  
AGCACCTGCCATACCCCTGCACAGTGCTGCGGCTGAGTTGGCTTCAAACCAGTGAGTT  
TTCTACCTCTACTATTGAAAGGGCACCTTGTCCACAGAACCAGAGTCTTGCCTGCATGT  
GG

## EXON 2

CTGAGGCCCTCTAGACATCCCTGTCCAGTCTTTTCAATCTCTGTGGGTTCTCATTTCTAGT  
CCAAGAGGGCCAGAAAGCAACCTGGAGGTTGAGAGCCAAAGAAAGCTGGAACCATGC  
TGACTTTGTAGACTGTGAGGACACAGAGTCTGTTCCTGGAAAGGCCAGTGTC AACGCA  
GATGAGGAAGTGGGAGGTCCCAATGTGCGCTGTATGTGGGGACAAGGCCACTGGC  
TATCACTTCAATGTGATGACATGTGAAGGATGCAAGGGCTTTTCAGGTAGAGTTACCC  
ATCAGCCTTCAGCCACGGTGGCAACCACTGAGCCAGTGGGTACATCTCAGGGCCT

## Exon 3

AGGTGGTATGGCCCGGAGCCCCAGGCCGAGGGCCCGGGCACCCGTGCATCCCCCCTT  
CTGCTCCCCATTCTCTCACAGGAGGGCCATGAAACGCAACGCCCGGCTGAGGTGCC  
CTTCCGGAAGGGCGCCTGCGAGATCACCCGGAAGACCCGGCGACAGTGCCAGGCCT  
GCCGCTGCGCAAGTGCTTGGAGAGCGGCATGAAGAAGGAGAGTGAGCAGTGGGCG  
CGCGGGCGGGCCGGCGCCGGGGTGCACGGCTCTGAGTAAGGACGTGCCGTGGGTGT  
GTGCATGCTTGTGTGGAGATGCGCGGCGAGTGTGCGCGTGAACACACGTGCACATGTG  
AGCT

## EXON 4

CTAACGGCTTCTGCTGCCCTTGAGAGGGTTACACAGTGGCTCTCCAGGGGGCTGGAGGC  
TCACCAGGGGCACGTGTGCCTGAGCCAGCCTCACTGTCCCTGCAGTGATCAT

11/12

Fig. 8 (continued)

GTCCGACGAGGCCGTGGAGGAGAGGCCGGCCTTGATCAAGCGGAAGAAAAGTGAAC  
GGACAGGGACTCAGCCACTGGGAGTGCAGGGGCTGACAGAGGAGCAGCGGATGATG  
ATCAGGGAGCTGATGGACGCTCAGATGAAAACCTTTGACACTACCTTCTCCCATTTC  
AGAATTTCCGGGTAGGAGGAACTGCACAGTGACCCGAGGTGTCAGTGCATCTTCATT  
CTCACATAGAACTGAGGTTCCCAAGGA

## Exon5/6

CTGAGTTGGGACCTGTCTATGAAAGCACATGCTGTCTCTCCTCTGTCCACCTCCTGGCA  
TGTGTCTCTAGCTGCCAGGGGTGCTTAGCAGTGGCTGCGAGTTGCCAGAGTCTCTGCA  
GGCCCCATCGAGGGAAGAAGCTGCCAAGTGGAGCCAGGTCCGGAAAGATCTGTGCT  
CTTTGAAGGTCTCTCTGCAGCTGCGGGGGGAGGATGGCAGTGTCTGGAACACTACAAAC  
CCCCAGCCGACAGTGGC→TGGGAAAGAGATCTTCTCCCTGCTGCCCCACATGGCTGA  
CATGTCAACCTACATGTTCAAAGGCATCATCAGCTTTGCCAAAGTCATCTCCTACTTCA  
GGTAGGACATGGAGACTGGGTGTTGGGTGTGGAAAGAACTGGAAGTGGCCAGGAG  
GTTCAAAGGGCCTGG

## EXONS 7&amp;8

CTGCTGGTGCCGGCCTGTGGGCTGCCTCCCAGGGAGCTGTCCTCCCCTCCCCATCCTT  
GCTGCCAGGGACTTGCCCATCGAGGACCAGATCTCCCTGCTGAAGGGGGCGGCTTTC  
GAGCTGTGTCAACTGAGATTCAACACAGTGTTCAACGCGGAGACTGGAACCTGGGAG  
TGTGGCCGGCTGTCTACTGCTTGAAGACACTGCAGGTGCCCGAGAGAGCCTGCCT  
GCCCTGGCAGAGGGAGGGAAACACTGCAGTTATGGGAGGAAGGGAGCTACGCCAGGA  
TATGCAGGTTCTGGGATGGCAGGGCAGGAAGATGGAATGGTGGAAAACAAGATATTGG  
TGAGGGATGATTAGATCTTGGTCAGCTTGCTGAGAAGCTGCCCCTCCATC→TCTGTTAC  
CATCCACAGGTGGCTTCCAGCAACTTCTACTGGAGCCCATGCTGAAATTCCACTACAT  
GCTGAAGAAGCTGCAGCTGCATGAGGAGGAGTATGTGCTGATGCAGGCCATCTCCCT  
CTTCTCCCCAGGTGAGGATCTCCCCTAGGCTGCCTGACATCCCCCCCAGCCTTATCTG  
CCCTCCCAGGGAAGGTCCCAGTC

## EXON 9

GAGCAATGCCCTGACTCTGGGCTGGACTGAGCTTGTCTTTGCCCCATGATCTTGCACCA  
CACCTCCCTCCCCTCCAGACCGCCCAGGTGTGCTGCAGCACCGCGTGGTGG

12/12

Fig. 8 (continued)

ACCAGCTGCAGGAGCAATTCGCCATTACTCTGAAGTCCTACATTGAATGCAATCGGCC  
CCAGCCTGCTCATAGGTGAGGACAGCAGGGGGGTGAGGACCCGTGAGGGTGATGTGAG  
GGAGCCGAGGTTTCAGGGAAATTCGCCAAGACTTCATGGCCAGAGGG

## EXON 9A

TGCTTGTGCAGCCTCAGAGCAGCCCTGAGGCTTGTGGGTCAGGGCGGGCTGCACCCA  
CAATCTTTTCTCTGGCTGGCATGCAGGTTCTTGTTCTGAAGATCATGGCTATGCTCAC  
CGAGCTCCGCAGCATCAATGCTCAGCACACCCAGCGGCTGCTGCGCATCCAGGACAT  
ACACCCCTTTGCTACGCCCCTCATGCAGGAGTTGTTTCGGCATCACAGGTAGCTGAGC  
GGCTGCCCTTGGGTGACACCTCCGAGAGGCAGCCAGACCCAGAGCCCTCTGAGCCG  
CCTCTCCCGGGCCAAGACAGATGGACACTGCCAAGAGC

EPO-Munich  
60  
10. Sep. 1999

## SEQUENCE LISTING

&lt;110&gt; EPIDAUROS Biotechnologie Aktiengesellschaft

<120> Polymorphisms in the human CYP3A4, CYP3A7 and hPXR  
genes and their use in diagnostic and therapeutic  
applications

&lt;130&gt; D2145EP

&lt;140&gt;

&lt;141&gt;

&lt;160&gt; 135

&lt;170&gt; PatentIn Ver. 2.1

&lt;210&gt; 1

&lt;211&gt; 20

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Description of Artificial Sequence: DNA

&lt;400&gt; 1

taatacgact cactataggg

20

&lt;210&gt; 2

&lt;211&gt; 18

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Description of Artificial Sequence: DNA

&lt;400&gt; 2

gaacccattc acatggac

18

&lt;210&gt; 3

&lt;211&gt; 18

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Description of Artificial Sequence: DNA

&lt;400&gt; 3

tgatcatgtc aggatctg

18

&lt;210&gt; 4

<211> 18  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: DNA

<400> 4  
ggccaacagc ctgtgctg 18

<210> 5  
<211> 18  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: DNA

<400> 5  
tccactgggtg aaaggttgg 18

<210> 16  
<211> 18  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: DNA

<400> 6  
gtgccatctc tatagctg 18

<210> 7  
<211> 18  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: DNA

<400> 7  
ctteccgcct cagatttc 18

<210> 8  
<211> 18  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: DNA



3 / 42

<400> 8  
gaaatctgag gcgggaag 18

<210> 9  
<211> 18  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: DNA

<400> 9  
gggtcttgat gattgttg 18

<210> 10  
<211> 18  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: DNA

<400> 10  
caacaatcca caagaccc 18

<210> 11  
<211> 18  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: DNA

<400> 11  
gtgtatcttc gaggcgac 18

<210> 12  
<211> 18  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: DNA

<400> 12  
ctttccattc ctcaccc 18

<210> 13

<211> 20  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: DNA

<400> 13  
cctttgtggg actcagtttc 20

<210> 14  
<211> 18  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: DNA

<400> 14  
gccactcaccctgatgtc 18

<210> 15  
<211> 18  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: DNA

<400> 15  
atcaccaccc accctttg 18

<210> 16  
<211> 18  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: DNA

<400> 16  
caaagggtgg gtggtgat 18

<210> 17  
<211> 18  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: DNA

5 / 42

<400> 17  
gagagcaaac ctcagcc

18

<210> 18  
<211> 18  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: DNA

<400> 18  
ggcatgaggt ttgctctc

18

<210> 19  
<211> 18  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: DNA

<400> 19  
ggtgccatcc cttgactc

18

<210> 20  
<211> 18  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: DNA

<400> 20  
gcagaggtgt gggccctg

18

<210> 21  
<211> 23  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: DNA

<400> 21  
ggagatcaag gaccacgctt gtg

23

<210> 22

<211> 24  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: DNA

<400> 22  
cttacgcttc tgccagtagc aacc 24

<210> 23  
<211> 20  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: DNA

<400> 23  
aacaggcgtg gaaacacaat 20

<210> 24  
<211> 18  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: DNA

<400> 24  
ctttcctgcc ctgcacag 18

<210> 25  
<211> 22  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: DNA

<400> 25  
cctctaactg ccagcaagtc tg 22

<210> 26  
<211> 21  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: DNA

7 / 42

<400> 26  
gcgctgagac tgtcctctgt g 21

<210> 27  
<211> 24  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: DNA

<400> 27  
agtctggctt cctgggttgg gctc 24

<210> 28  
<211> 26  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: DNA

<400> 28  
gaagtggacg tggaaccttc ctggac 26

<210> 29  
<211> 20  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: DNA

<400> 29  
ctacaacctt ggagacctcc 20

<210> 30  
<211> 20  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: DNA

<400> 30  
tacctgtccc caccagattc 20

<210> 31

8 / 42

<211> 23  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: DNA

<400> 31  
gtctgtcttg actggacatg tgg 23

<210> 32  
<211> 22  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: DNA

<400> 32  
gatgatggtc acacatatctatc 22

<210> 33  
<211> 23  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: DNA

<400> 33  
ggagatcaag gaccacgctt gtc 23

<210> 34  
<211> 25  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: DNA

<400> 34  
ctcatcatedtgggaatacttcctgc 25

<210> 35  
<211> 21  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: DNA

<400> 35  
ccagtatgag ttgttctctg g 21

<210> 36  
<211> 24  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: DNA

<400> 36  
aggcagaata tgcttgaacc aggc 24

<210> 37  
<211> 18  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: DNA

<400> 37  
tcatgtccga cgaggccg 18

<210> 38  
<211> 18  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: DNA

<400> 38  
cccacatggc tgacatgt 18

<210> 39  
<211> 18  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: DNA

<400> 39  
cccacgagg accagatc 18

<210> 40

<211> 18  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: DNA

<400> 40  
gtcttccaag cagtagga 18

<210> 41  
<211> 18  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: DNA

<400> 41  
cagcatgggc tccagtag 18

<210> 42  
<211> 18  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: DNA

<400> 42  
cctgtgatgc cgaacaac 18

<210> 43  
<211> 18  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: DNA

<400> 43  
cattgaatgc aatcggcc 18

<210> 44  
<211> 18  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: DNA



11 / 42

<400> 44  
gctcttggca gtgtccat

18

<210> 45  
<211> 18  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: DNA

<400> 45  
ggaaagccca gtgtcaac

18

<210> 46  
<211> 18  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: DNA

<400> 46  
ccatgaaacg caacgccc

18

<210> 47  
<211> 18  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: DNA

<400> 47  
ccttgcatcc ttcacatg

18

<210> 48  
<211> 18  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: DNA

<400> 48  
catgccgctc tccaggca

18

<210> 49

12 / 42

<211> 18  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: DNA

<400> 49  
cggcctcgtc ggacatga

18

<210> 50  
<211> 18  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: DNA

<400> 50  
acatgtcagc catgtggg

18

<210> 51  
<211> 20  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: DNA

<400> 51  
caagccaagt gttcacagt

20

<210> 52  
<211> 20  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: DNA

<400> 52  
cactgtgaac acttggett

20

<210> 53  
<211> 24  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: DNA

13 / 42

<400> 53  
caaggacagc agcatgacag tcac

24

<210> 54  
<211> 20  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: DNA

<400> 54  
agccaactca gccgcagc

18

<210> 55  
<211> 12  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: DNA

<400> 55  
aagcaggtat gg

12

<210> 56  
<211> 12  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: DNA

<400> 56  
aaaccagtga gt

12

<210> 57  
<211> 12  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: DNA

<400> 57  
ttctagtcca ag

12

<210> 58

14 / 42

<211> 12  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: DNA

<400> 58  
tttcaggtag ag 12

<210> 59  
<211> 12  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: DNA

<400> 59  
tcacaggagggc 12

<210> 60  
<211> 12  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: DNA

<400> 60  
aggagagtga gc 12

<210> 61  
<211> 12  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: DNA

<400> 61  
ctgcagtgatcca 12

<210> 62  
<211> 12  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: DNA

15 / 42

<400> 62  
ttccgggtag ga

12

<210> 63  
<211> 12  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: DNA

<400> 63  
tcctagctgc ca

12

<210> 64  
<211> 12  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: DNA

<400> 64  
cttcaggtag ga

12

<210> 65  
<211> 12  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: DNA

<400> 65  
tgccagggac tt

12

<210> 66  
<211> 12  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: DNA

<400> 66  
ctgcaggtgc cc

12

<210> 67

<211> 12  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: DNA

---

<400> 67  
ccacaggtgg ct 12

<210> 68  
<211> 12  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: DNA

<400> 68  
ccccaggtga gg 12

<210> 69  
<211> 12  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: DNA

<400> 69  
ctccagaccg cc 12

<210> 70  
<211> 12  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: DNA

<400> 70  
tcataggtga gc 12

---

<210> 71  
<211> 12  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: DNA

<400> 71  
atgcaggttc tt

12

<210> 72  
<211> 20  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: DNA

<400> 72  
tcaagtgctg gacttgggac

20

<210> 73  
<211> 20  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: DNA

<400> 73  
cccactatga tgctgacctc

20

<210> 74  
<211> 18  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: DNA

<400> 74  
ctgaggcctc tacacatc

18

<210> 75  
<211> 18  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: DNA

<400> 75  
aggccctgag atgttacc

18

<210> 76

<211> 18  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: DNA

---

<400> 76  
ctgagttggg acctgtct 18

<210> 77  
<211> 18  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: DNA

<400> 77  
ccaggccctt tgaacctc 18

<210> 78  
<211> 18  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: DNA

<400> 78  
ctgctggtgc cggcctgt 18

<210> 79  
<211> 18  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: DNA

<400> 79  
gactgggacc ttccctgg 18

---

<210> 80  
<211> 18  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: DNA



19 / 42

<400> 80  
gagcaatgcc ctgactct 18

<210> 81  
<211> 18  
<212> DNA  
<213> Artificial Sequence  
  
<220>  
<223> Description of Artificial Sequence: DNA

<400> 81  
ccctctggcc atgaagtc 18

<210> 82  
<211> 18  
<212> DNA  
<213> Artificial Sequence  
  
<220>  
<223> Description of Artificial Sequence: DNA

<400> 82  
tgcttgtgca gcctcaga 18

<210> 83  
<211> 18  
<212> DNA  
<213> Artificial Sequence  
  
<220>  
<223> Description of Artificial Sequence: DNA

<400> 83  
gctcttggca gtgtccat 18

<210> 84  
<211> 15  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: DNA

<400> 84  
tcccagggt tttgt 15

<210> 85

20 / 42

<211> 15  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: DNA

---

<400> 85  
acaaaagccc tggga 15

<210> 86  
<211> 15  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: DNA

<400> 86  
tcccaggact tttgt 15

<210> 87  
<211> 15  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: DNA

<400> 87  
acaaaagtcc tggga 15

<210> 88  
<211> 14  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: DNA

<400> 88  
tatctttctc tctt 14

---

<210> 89  
<211> 14  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: DNA

21 / 42

<400> 89  
aagagagaaa gat

13

<210> 90  
<211> 14  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: DNA

<400> 90  
tatcttgctc tctt

14

<210> 91  
<211> 14  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: DNA

<400> 91  
aagagagcaa gata

14

<210> 92  
<211> 13  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: DNA

<400> 92  
tactggacag agc

13

<210> 93  
<211> 13  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: DNA

<400> 93  
gctctgtcca gta

13

<210> 94

22 / 42

&lt;211&gt; 13

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Description of Artificial Sequence: DNA

&lt;400&gt; 94

tactggagag agc

13

&lt;210&gt; 95

&lt;211&gt; 13

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Description of Artificial Sequence: DNA

&lt;400&gt; 95

gctctctcca gta

13

&lt;210&gt; 96

&lt;211&gt; 14

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Description of Artificial Sequence: DNA

&lt;400&gt; 96

cctgaacaag gcag

14

&lt;210&gt; 97

&lt;211&gt; 14

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Description of Artificial Sequence: DNA

&lt;400&gt; 97

ctgccttgta cagga

14

&lt;210&gt; 98

&lt;211&gt; 14

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Description of Artificial Sequence: DNA

23 / 42

<400> 98  
cctgaaaaag gcag

14

<210> 99  
<211> 14  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: DNA

<400> 99  
ctgccttttt cagg

14

<210> 100  
<211> 13  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: DNA

<400> 100  
cagtggcggg aaa

13

<210> 101  
<211> 13  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: DNA

<400> 101  
tttcccggca ctg

13

<210> 102  
<211> 13  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: DNA

<400> 102  
cagtgggtggg aaa

13

<210> 103

<211> 13  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: DNA

---

<400> 103  
tttcccacca ctg 13

<210> 104  
<211> 15  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: DNA

<400> 104  
cctccatcctgttac 15

<210> 105  
<211> 15  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: DNA

<400> 105  
gtaacaggat ggagg 15

<210> 106  
<211> 15  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: DNA

<400> 106  
cctccattctgttac 15

---

<210> 107  
<211> 15  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: DNA

25 / 42

<400> 107  
gtaacagaat ggagg

15

<210> 108  
<211> 249  
<212> DNA  
<213> Homo sapiens

<220>  
<221> intron  
<222> (1)..(115)

<220>  
<221> exon  
<222> (116)..(168)

<220>  
<221> intron  
<222> (169)..(249)

<220>  
<221> CDS  
<222> (116)..(166)

<400> 108  
cctctaactg ccagcaagtc tgatttcatt ggcttcgact gttttcatcc caattagagg 60  
cagggttaag tacattaataa ataataatca aatattatatt tgtttctcct ccag grc 118  
Xaa  
1

ttt tgt atg ttt gac atg gaa tgt cat aaa aag tat gga aaa gtg tgg 166  
Phe Cys Met Phe Asp Met Glu Cys His Lys Lys Tyr Gly Lys Val Trp  
5 10 15

gggtgagtat tctggaaact tccattggat agacttggtt ctatgatgag tttacccac 226  
tgcacagagg acagtctcag ccc  
249

<210> 109  
<211> 17  
<212> PRT  
<213> Homo sapiens

<400> 109  
Xaa Phe Cys Met Phe Asp Met Glu Cys His Lys Lys Tyr Gly Lys Val  
1 5 10 15  
Trp

<210> 110  
 <211> 293  
 <212> DNAA  
 <213> Homo sapiens

<220>  
 <221> intron  
 <222> (1)..(77)

<220>  
 <221> exon  
 <222> (78)..(177)

<220>  
 <221> intron  
 <222> (178)..(293)

<220>  
 <221> CDS  
 <222> (79)..(177)

<400> 110  
 agtctggcct cctggggttg gctcagctg tagaataagg ctgttgatgt attaatcaact 60

ctgttttttt cacacagc ttttat gatgggcaa cagcctgtg ctg gct ate 111  
 Phe Tyr Asp Gly Gln Gln Pro Val Leu Ala Ile  
 1 5 10

aca gat cct gac atg atc aaa aca gtg cta gtg aaa gaa tgt tat tct 159  
 Thr Asp Pro Asp Met Ile Lys Thr Val Leu Val Lys Glu Cys Tyr Ser  
 15 20 25

gtc ttc aca aac cgg agg gtaagcattc atgtgttgaa attaaaatac 207  
 Val Phe Thr Asn Arg Arg  
 30

tgattgatta aatttatatt ttgaaattct tatatattca tagacagttg cctaaaaaat 267  
 gtccaggaag gttccacgtc cacttc 293

<210> 111  
 <211> 33  
 <212> PRT  
 <213> Homo sapiens

<400> 111  
 Phe Tyr Asp Gly Gln Gln Pro Val Leu Ala Ile Thr Asp Pro Asp Met  
 1 5 10 15

Ile Lys Thr Val Leu Val Lys Glu Cys Tyr Ser Val Phe Thr Asn Arg  
 20 25 30



27 / 42

Arg

<210> 112  
 <211> 236  
 <212> DNA  
 <213> Homo sapiens

<220>  
 <221> intron  
 <222> (1)..(61)

<220>  
 <221> exon  
 <222> (62)..(175)

<220>  
 <221> intron  
 <222> (176)..(236)

<220>  
 <221> CDS  
 <222> (62)..(175)

<400> 112  
 ctacaacccat ggagacctcc acaactgatg taggacaaaa tgtttctgct ttgaactcta 60  
 g cct ttt ggt cca gtg gga ttt atg aaa agt gcc atc tct ata gct gag 109  
 Pro Phe Gly Pro Val Gly Phe Met Lys Ser Ala Ile Ser Ile Ala Glu  
 1 5 10 15  
 gat gaa gaa tgg aag aga tta cga tca ttg ctg tct cca acc ttc acc 157  
 Asp Glu Glu Trp Lys Arg Leu Arg Ser Leu Leu Ser Pro Thr Phe Thr  
 20 25 30  
 agt gga aaa ctc aag gag gtatgaaaat aacatgagtt ttaataagaa 205  
 Ser Gly Lys Leu Lys Glu  
 35  
 acttaaagaa tgaatctggt ggggacaggt a 236

<210> 113  
 <211> 38  
 <212> PRT  
 <213> Homo sapiens

<400> 113  
 Pro Phe Gly Pro Val Gly Phe Met Lys Ser Ala Ile Ser Ile Ala Glu  
 1 5 10 15

Asp Glu Glu Trp Lys Arg Leu Arg Ser Leu Leu Ser Pro Thr Phe Thr  
 20 25 30

Ser Gly Lys Leu Lys Glu  
 35

<210> 114  
 <211> 393  
 <212> DNA  
 <213> Homo sapiens

<220>  
 <221> intron  
 <222> (1)..(98)

<220>  
 <221> exon  
 <222> (99)..(246)

<220>  
 <221> intron  
 <222> (248)..(393)

<220>  
 <221> CDS  
 <222> (100)..(246)

<400> 114  
 gtctgtcttg actggacatg tggctttcct gatgcacgca tagaggaagg atggtaaaaa 60  
 ggtgctgatt ttaattttcc acatctttct ccactcagc gtc ttt ggg gcc tac 114  
 Val Phe Gly Ala Tyr  
 1 5

agc atg gat gtg atc act agc aca tca ttt gga gtg aac atc gac tct 162  
 Ser Met Asp Val Ile Thr Ser Thr Ser Phe Gly Val Asn Ile Asp Ser  
 10 15 20

ctc aac aat cca caa gac ccc ttt gtg gaa aac acc aag aag ctt tta 210  
 Leu Asn Asn Pro Gln Asp Pro Phe Val Glu Asn Thr Lys Lys Leu Leu  
 25 30 35

aga ttt gat ttt ttg gat cca ttc ttt ctctcaata agtatgtgga 256  
 Arg Phe Asp Phe Leu Asp Pro Phe Phe Leu Ser Ile  
 40 45

ctactatttc cttttattta tcttyctctc ttaaaaataa ctgctttatt gagatataaa 316  
 tcaccatgta attcatccac ttaaaatata cagttcagtg attttagta catttgaaga 376  
 tatgtgtgac catcatc 393

29 / 42

<210> 115  
 <211> 49  
 <212> PRT  
 <213> Homo sapiens

<400> 115  
 Val Phe Gly Ala Tyr Ser Met Asp Val Ile Thr Ser Thr Ser Phe Gly  
           1                  5                  10                  15  
 Val Asn Ile Asp Ser Leu Asn Asn Pro Gln Asp Pro Phe Val Glu Asn  
                   20                  25                  30  
 Thr Lys Lys Leu Leu Arg Phe Asp Phe Leu Asp Pro Phe Phe Leu Ser  
           35                  40                  45  
 Ile

<210> 116  
 <211> 240  
 <212> DNA  
 <213> Homo sapiens

<220>  
 <221> intron  
 <222> (1)..(82)

<220>  
 <221> exon  
 <222> (83)..(149)

<220>  
 <221> intron  
 <222> (150)..(240)

<220>  
 <221> CDS  
 <222> (83)..(148)

<400> 116  
 ggagatcaag gaccacgctt gtgatttact tctgacttca ggagccactt tctgtcagtg 60  
 aaatttctct ttttgcttct ag cac cga gtg gat ttc ctt cag ctg atg att 112  
                                   His Arg Val Asp Phe Leu Gln Leu Met Ile  
                                   1                  5                  10

gac tct cag aat tca aaa gaa act gag tcc cac aaa ggtaaccaga 158  
 Asp Ser Gln Asn Ser Lys Glu Thr Glu Ser His Lys  
                   15                  20

gtgtttctga gggctacttg tggggcactc agagggaagg ccttggttctg aaaatgtgca 218



31 / 42

tgc aaa aaa gat gtt gaa atc aat ggg atg ttt att ccc aaa ggg gtg 261  
 Cys Lys Lys Asp Val Glu Ile Asn Gly Met Phe Ile Pro Lys Gly Val  
 35 40 45 50

gtg gtg atg att cca agc tat gtt ctt cat cat gac cca aag tac tgg 309  
 Val Val Met Ile Pro Ser Tyr Val Leu His His Asp Pro Lys Tyr Trp  
 55 60 65

asa gag cct gag aag ttc ctc cct gaa aggtaggagg cccctgggaa 356  
 Xaa Glu Pro Glu Lys Phe Leu Pro Glu  
 70 75

gggagccctc cctgaaccag cctggttcaa gcatattctg cct 399

<210> 119  
 <211> 75  
 <212> PRT  
 <213> Homo sapiens

<400> 119  
 Ala Pro Pro Thr Tyr Asp Thr Val Leu Gln Leu Glu Tyr Leu Asp Met  
 1 5 10 15

Val Val Asn Glu Thr Leu Arg Leu Phe Pro Val Ala Met Arg Leu Glu  
 20 25 30

Arg Val Cys Lys Lys Asp Val Glu Ile Asn Gly Met Phe Ile Pro Lys  
 35 40 45

Gly Val Val Val Met Ile Pro Ser Tyr Val Leu His His Asp Pro Lys  
 50 55 60

Tyr Trp Xaa Glu Pro Glu Lys Phe Leu Pro Glu  
 65 70 75

<210> 120  
 <211> 962  
 <212> DNA  
 <213> Homo sapiens

<220>  
 <221> intron  
 <222> (1)..(66)

<220>  
 <221> intron  
 <222> (348)..(750)

<220>  
 <221> exon  
 <222> (751)..(894)

<220>  
<221> intron  
<222> (895)..(962)

<220>  
<221> CDS  
<222> (800) .. (892)

<400> 120  
tcaagtgctg gacttgggac ttaggagggg caatggagcc gcttagtgcc tacatctgac 60  
ttggactgaa atatatgtga gagacaagat tgtctcatat ccggggaaat cataacctat 120  
gactaggacg ggaagaggaa gcaactgcctt tacttcagtg ggaatctcgg cctcagcctg 180  
caagccaagt gttcacagtg aaaaaagcaa gagaataagc taatactcct gtcctgaaca 240  
aggcagcggc tccttggtaa agcactcctt tgatcgatcc ttgacccg attgttcaaa 300  
gtggacccca ggggagaaagtcggagcaaaagaaacttaccac caagcaggta tgggtttttt 360  
ttctttctcttttggctggggggtgacccgacttcagctcc agccaaaagatgtgtgtgaa 420  
cacaatatat ccttctgttt gaggtcagca tcatagtggg tctgtaatca ttgttggcctt 480  
gctgctgtct cctcattttt aggggtgaaaaa aaaaaagca tgaaaacaat cacttaattgt 540  
tgagcccat tactgatgcttctctgggtcct gcaactagcct cctagaaaaat caccacagc 600  
cttaactact gcatgagtta ccacaagtca cacataaac cagctccctg ttacagggtt 660  
ggagtccctg gaccagga ataccacctc caaggactgt gggagctggg gactatggga 720  
actgggatca actcagtcct gattcctttt ggctgctgg gttagtgtg gcagccccc 780  
tgaggccaag gacagcagc atg aca gtc acc agg act cac cac ttc aag gag 832  
Met Thr Val Thr Arg Thr His His Phe Lys Glu  
1 5 10  
ggg tcc ctc aga gca cct gcc ata ccc ctg cac agt gct gcg gct gag 880  
Gly Ser Leu Arg Ala Pro Ala Ile Pro Leu His Ser Ala Ala Ala Glu  
15 20 25  
ttg gct tca aac cagtgagttt tctacctctatctattgaag ggcacctgt 932  
Leu Ala Ser Asn  
30

cccacagaac cgagtcttgc ctgcatgtgg

```
<210> 121
<211> 31
<212> PRT
```

33 / 42

&lt;213&gt; Homo sapiens

&lt;400&gt; 121

Met Thr Val Thr Arg Thr His His Phe Lys Glu Gly Ser Leu Arg Ala  
 1 5 10 15

~~Pro Ala Ile Pro Leu His Ser Ala Ala Ala Glu Leu Ala Ser Asn~~  
 20 25 30

&lt;210&gt; 122

&lt;211&gt; 345

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; intron

&lt;222&gt; (1)..(60)

&lt;220&gt;

&lt;221&gt; exon

&lt;222&gt; (61)..(279)

&lt;220&gt;

&lt;221&gt; intron

&lt;222&gt; (280)..(345)

&lt;220&gt;

&lt;221&gt; CDS

&lt;222&gt; (62)..(277)

&lt;400&gt; 122

ctgaggcctc tacacatccc tgtccagtct tttcattctc tgtgggtttc tcatttctag 60

t cca aga ggc cca gaa gca aac ctg gag gtg aga ccc aaa gaa agc tgg 109  
 Pro Arg Gly Pro Glu Ala Asn Leu Glu Val Arg Pro Lys Glu Ser Trp  
 1 5 10 15

aac cat gct gac ttt gta cac tgt gag gac aca gag tct gtt cct gga 157  
 Asn His Ala Asp Phe Val His Cys Glu Asp Thr Glu Ser Val Pro Gly  
 20 25 30

aag ccc agt gtc aac gca gat gag gaa gtc gga ggt ccc caa atc tgc 205  
 Lys Pro Ser Val Asn Ala Asp Glu Glu Val Gly Gly Pro Gln Ile Cys  
 35 40 45

cgt gta tgt ggg gac aag gcc act ggc tat cac ttc aat gtc atg aca 253  
 Arg Val Cys Gly Asp Lys Ala Thr Gly Tyr His Phe Asn Val Met Thr  
 50 55 60

tgt gaa gga tgc aag ggc ttt ttc aggtagagtt acccatcagc cttcacccac 307  
 Cys Glu Gly Cys Lys Gly Phe Phe  
 65 70

gtgccaccac.tgacccactg.ggtaacatct.cagggcct

345

&lt;210&gt; 123

&lt;211&gt; 72

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 123

Pro Arg Gly Pro Glu Ala Asn Leu Glu Val Arg Pro Lys Glu Ser Trp  
1 5 10 15

Asn His Ala Asp Phe Val His Cys Glu Asp Thr Glu Ser Val Pro Gly  
20 25 30

Lys Pro Ser Val Asn Ala Asp Glu Glu Val Gly Gly Pro Gln Ile Cys  
35 40 45

Arg Val Cys Gly Asp Lys Ala Thr Gly Tyr His Phe Asn Val Met Thr  
50 55 60

Cys Glu Gly Cys Lys Gly Phe Phe  
65 70

&lt;210&gt; 124

&lt;211&gt; 344

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; intron

&lt;222&gt; (1)..(78)

&lt;220&gt;

&lt;221&gt; exon

&lt;222&gt; (79)..(212)

&lt;220&gt;

&lt;221&gt; intron

&lt;222&gt; (213)..(344)

&lt;220&gt;

&lt;221&gt; CDS

&lt;222&gt; (79)..(210)

&lt;400&gt; 124

agtggtatg gcccgagacc ccaggccgag ggcccgaggc cccgtgcatc cccctttctg 60

ctccccattc totcacag gag ggc cat gaa acg caa cgc ccg gct gag gtg 111  
Glu Gly His Glu Thr Gln Arg Pro Ala Glu Val  
1 5 10



35 / 42

```

ccc ctt ccg gaa ggg cgc ctg cga gat cac ccg gaa gac ccg gcg aca 159
Pro Leu Pro Glu Gly Arg Leu Arg Asp His Pro Glu Asp Pro Ala Thr
          15                20                25

```

```

gtg cca ggc ctg ccg cct gcg caa gtg cct gga gag cgg cat gaa gaa 207
Val Pro Gly Leu Pro Pro Ala Gln Val Pro Gly Glu Arg His Glu Glu
          30                35                40

```

```

gga gaggtagcag tgggcgcgcg ggcggggccgg cgccgggggtg cacggctctg 260
Gly

```

```

agtaaggacg tgccgtgggt gtgtgcatgc ttgtgtggag atgcgcgccg agtgtgcgcg 320

```

```

tgaacacacg tgcacatgtg agct 344

```

&lt;210&gt; 125

&lt;211&gt; 44

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 125

```

Glu Gly His Glu Thr Gln Arg Pro Ala Glu Val Pro Leu Pro Glu Gly
  1              5              10              15

```

```

Arg Leu Arg Asp His Pro Glu Asp Pro Ala Thr Val Pro Gly Leu Pro
          20                25                30

```

```

Pro Ala Gln Val Pro Gly Glu Arg His Glu Glu Gly
          35                40

```

&lt;210&gt; 126

&lt;211&gt; 367

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; intron

&lt;222&gt; (1)..(103)

&lt;220&gt;

&lt;221&gt; exon

&lt;222&gt; (104)..(291)

&lt;220&gt;

&lt;221&gt; intron

&lt;222&gt; (292)..(367)

&lt;220&gt;

&lt;221&gt; CDS

&lt;222&gt; (106)..(291)

<400> 126  
 ctaacggccttctgctgcctt gagaggggtta cacagtggcttctccagggggctggagggctc 60  
 accagggggcgcgtgctgcctg agccagcctc actgtccctgkcagtg atc atg atc cagac 117  
 Ile Met Ser Asp

gag gcc gtg gag gag agg cgg gcc ttg atc aag cgg aag aaa agt gaa 165  
 Glu Ala Val Glu Glu Arg Arg Ala Leu Ile Lys Arg Lys Lys Ser Glu 20  
 5 10 15  
 cgg aca ggg act cag cca ctg gga gtg cag ggg ctg aca gag gag cag 213  
 Arg Thr Gly Thr Gln Pro Leu Gly Val Gln Gly Leu Thr Glu Glu Gln 35  
 25 30  
 cgg atg atg atc agg gag ctg atg gac gct cag atg aaa acc ttt gac 261  
 Arg Met Met Ile Arg Glu Leu Met Asp Ala Gln Met Lys Thr Phe Asp 50  
 40 45  
 act acc ttc tcc cat ttc aag aat ttc cgg gtaggaggaactgcacagtg 314  
 Thr Thr Phe Ser His Phe Lys Asn Phe Arg 60  
 55  
 acccgagggtg tcactgcca tctcattctc acatagaaac tgaggttccc caagga 367  
 60

<210> 127  
 <211> 62  
 <212> PRT  
 <213> Homo sapiens

<400> 127  
 Ile Met Ser Asp Glu Ala Val Glu Glu Arg Arg Ala Leu Ile Lys Arg  
 1 5 10 15  
 Lys Lys Ser Glu Arg Thr Gly Thr Gln Pro Leu Gly Val Gln Gly Leu  
 20 25 30  
 Thr Glu Glu Gln Arg Met Met Ile Arg Glu Leu Met Asp Ala Gln Met  
 35 40 45  
 Lys Thr Phe Asp Thr Thr Phe Ser His Phe Lys Asn Phe Arg  
 50 55 60

<210> 128  
 <211> 415  
 <212> DNA  
 <213> Homo sapiens

<220>  
 <221> intron

<222> (1) .. (69)

<220>

<221> exon

<222> (70) .. (344)

 $\langle 220 \rangle$ 

<221> intron

$\langle 222 \rangle \quad (345) \dots (415)$

<220>

<221> CDS

$\langle 222 \rangle \quad (70) \dots (342)$

<400> 128

ctgagttggg acctgtctat gaaagcacat gctgtctctc ctctgtccac ctctgggcat 60

gtgtcctag ctg cca ggg gtg ctt agc agt ggc tgc gag ttg cca gag tct 111  
Leu Pro Gly Val Leu Ser Ser Gly Cys Glu Leu Pro Glu Ser  
1 5 10

ctg cag gcc cca tcg agg gaa gaa gct gcc aag tgg agc cag gtc cgg 159  
Leu Gln Ala Pro Ser Arg Glu Glu Ala Ala Lys Trp Ser Gln Val Arg  
15 20 25 30

aaa gat ctg tgc tct ttg aag gtc tct ctg cag ctg cgg ggg gag gat 207  
Lys Asp Leu Cys Ser Leu Lys Val Ser Leu Gln Leu Arg Gly Glu Asp  
35 40 45

ggc agt gtc tgg aac tac aaa ccc cca gcc gac agt ggy ggg aaa gag 255  
Gly Ser Val Trp Asn Tyr Lys Pro Pro Ala Asp Ser Xaa Gly Lys Glu  
50 55 60

atc ttc tcc ctg ctg ccc cac atg gct gac atg tca acc tac atg ttc 303  
Ile Phe Ser Leu Leu Pro His Met Ala Asp Met Ser Thr Tyr Met Phe  
65 70 75

aaa ggc atc atc agc ttt gcc aaa gtc atc tcc tac ttc aggtaggaca 352  
Lys Gly Ile Ile Ser Phe Ala Lys Val Ile Ser Tyr Phe  
80 85 90

tggagactgg gtggttgggt gtggaaaaga actggaagtg gccaggaggt tcaaagggcc 412  
tgg 415

<210> 129

<211> 91

<212> PRT

<213> Homo sapiens

<400> 129

Leu Pro Gly Val Leu Ser Ser Gly Cys Glu Leu Pro Glu Ser Leu Gln  
1 5 10 15

Ala Pro Ser Arg Glu Glu Ala Ala Lys Trp Ser Gln Val Arg Lys Asp  
20 25 30

Leu Cys Ser Leu Lys Val Ser Leu Gln Leu Arg Gly Glu Asp Gly Ser  
35 40 45

Val Trp Asn Tyr Lys Pro Pro Ala Asp Ser Xaa Gly Lys Glu Ile Phe  
50 55 60

Ser Leu Leu Pro His Met Ala Asp Met Ser Thr Tyr Met Phe Lys Gly  
65 70 75 80

Ile Ile Ser Phe Ala Lys Val Ile Ser Tyr Phe  
85 90

<210> 130  
<211> 598  
<212> DNA  
<213> Homo sapiens

<220>  
<221> intron  
<222> (1) .. (66)

<220>  
<221> exon  
<222> (67) .. (209)

<220>  
<221> intron  
<222> (210) .. (410)

<220>  
<221> exon  
<222> (411) .. (527)

<220>  
<221> intron  
<222> (528) .. (598)

<220>  
<221> CDS  
<222> (68) .. (209)

<220>  
<221> CDS  
<222> (411) .. (526)

<400> 130  
ctgctgtg gc cggcctgtgg gctgcctccc agggagctgt cctcccctcc ccataccttgc 60

39 / 42

tgccagg gac ttg ccc atc gag gac cag atc tcc ctg ctg aag ggg gcc 109  
 Asp Leu Pro Ile Glu Asp Gln Ile Ser Leu Leu Lys Gly Ala  
 1 5 10

gct ttc gag ctg tgt caa ctg aga ttc aac aca gtg ttc aac gcg gag 157  
 Ala Phe Glu Leu Cys Gln Leu Arg Phe Asn Thr Val Phe Asn Ala Glu  
 15 20 25 30

act gga acc tgg gag tgt ggc cgg ctg tcc tac tgc ttg gaa gac act 205  
 Thr Gly Thr Trp Glu Cys Gly Arg Leu Ser Tyr Cys Leu Glu Asp Thr  
 35 40 45

gca ggt gcccgagaga gcctgcctgc cctggcagag ggagggaaac actgcagtta 261  
 Ala

tgggaggaag ggagctacgc caggatatgc aggttctggg atggcagggc aggaagatgg 321

aatggtggaa aacaagatat tgggtgagga tgattagatc ttggtcagct tgctgagaag 381

ctgccccctcc atyctgttac catccacagg t ggc ttc cag caa ctt cta ctg 433  
 Gly Phe Gln Gln Leu Leu Leu  
 50 55

gag ccc atg ctg aaa ttc cac tac atg ctg aag aag ctg cag ctg cat 481  
 Glu Pro Met Leu Lys Phe His Tyr Met Leu Lys Lys Leu Gln Leu His  
 60 65 70

gag gag gag tat gtg ctg atg cag gcc atc tcc ctc ttc tcc cca 526  
 Glu Glu Glu Tyr Val Leu Met Gln Ala Ile Ser Leu Phe Ser Pro  
 75 80 85

ggtgaggatc tcccctagga tgccctgacat cccccccagc cttatctgcc ctccccaggg 586

aaggtcccag tc 598

<210> 131

<211> 86

<212> PRT

<213> Homo sapiens

<400> 131

Asp Leu Pro Ile Glu Asp Gln Ile Ser Leu Leu Lys Gly Ala Ala Phe  
 1 5 10 15

Glu Leu Cys Gln Leu Arg Phe Asn Thr Val Phe Asn Ala Glu Thr Gly  
 20 25 30

Thr Trp Glu Cys Gly Arg Leu Ser Tyr Cys Leu Glu Asp Thr Ala Gly  
 35 40 45

Gly Phe Gln Gln Leu Leu Leu Glu Pro Met Leu Lys Phe His Tyr Met  
 50 55 60

Leu Lys Lys Leu Gln Leu His Glu Glu Glu Tyr Val Leu Met Gln Ala  
 65 70 75 80  
 Ile Ser Leu Phe Ser Pro  
 85

<210> 132  
 <211> 271  
 <212> DNA  
 <213> Homo sapiens

<220>  
 <221> intron  
 <222> (1)..(77)

<220>  
 <221> exon  
 <222> (78)..(183)

<220>  
 <221> intron  
 <222> (184)..(271)

<220>  
 <221> CDS  
 <222> (80)..(181)

<400> 132  
 gagcaatgcc ctgactctgg gctggactga gcttgtcttt gcccatgat cttgcaccac 60

acctccctcc cctccagac cgc cca ggt gtg ctg cag cac cgc gtg gtg gac 112  
 Arg Pro Gly Val Leu Gln His Arg Val Val Asp  
 1 5 10

cag ctg cag gag caa ttc gcc att act ctg aag tcc tac att gaa tgc 160  
 Gln Leu Gln Glu Gln Phe Ala Ile Thr Leu Lys Ser Tyr Ile Glu Cys  
 15 20 25

aat cgg ccc cag cct gct cat aggtgagcac agcagggggt gaggaccgt 211  
 Asn Arg Pro Gln Pro Ala His  
 30

gagggtagtg tgagggagcc gaggttcagg gaaattgcc aagacttcac aggccagaggg 271

<210> 133  
 <211> 34  
 <212> PRT  
 <213> Homo sapiens

<400> 133  
 Arg Pro Gly Val Leu Gln His Arg Val Val Asp Gln Leu Gln Glu Gln

1                      5                      10                      15  
Phe Ala Ile Thr Leu Lys Ser Tyr Ile Glu Cys Asn Arg Pro Gln Pro  
                        20                      25                      30

Ala His

```
<210> 134
<211> 324
<212> DNA
<213> Homo sapiens
```

```
<220>
<221> intron
<222> (1)..(83)
```

```
<220>
<221> exon
<222> (84) .. (224)
```

```
<220>
<221> 3'UTR
<222> (226)..(324)
```

```
<220>
<221> CDS
<222> (85) .. (225)
```

<400> 134  
tgcttgtgca gcctcagagc agccctgagg cttgtgggtc agggcgggct gcaccacaa 60

tcttttctct ggctggcatg cagg ttc ttg ttc ctg aag atc atg gct atg 111  
Phe Leu Phe Leu Lys Ile Met Ala Met  
1 5

ctc acc gag ctc cgc agc atc aat gct cag cac acc cag cgg ctg ctg 159  
Leu Thr Glu Leu Arg Ser Ile Asn Ala Gln His Thr Gln Arg Leu Leu  
10 15 20 25

cgc atc cag gac ata cac ccc ttt gct acg ccc ctc atg cag gag ttg 207  
Arg Ile Gln Asp Ile His Pro Phe Ala Thr Pro Leu Met Gln Glu Leu  
30 35 40

ttc ggc atc aca ggt agc tgagcggctg cccttggggtg acacctccga 255  
Phe Gly Ile Thr Gly Ser  
45

gaggcagcca gaccagagc cctctgagcc gccactcccg ggccaagaca gatggacact 315  
gccaagagc 324

10-09-1999

EP09118120.7

SEQ

42 / 42

<210> 135

<211> 47

<212> PRT

<213> Homo sapiens

<400> 135

Phe Leu Phe Leu Lys Ile Met Ala Met Leu Thr Glu Leu Arg Ser Ile  
1 5 10 15

Asn Ala Gln His Thr Gln Arg Leu Leu Arg Ile Gln Asp Ile His Pro  
20 25 30

Phe Ala Thr Pro Leu Met Gln Glu Leu Phe Gly Ile Thr Gly Ser  
35 40 45

Printed:13-12-2000



10. Sep. 1999

**Abstract**

Described are general means and methods of diagnosing and treating the phenotypic spectrum as well as the overlapping clinical characteristics with several forms of inherited abnormal expression and/or function of the CYP3A4, CYP3A7 and hPXR genes. In particular, polynucleotides of molecular variant CYP3A4, CYP3A7 and hPXR genes which, for example, are associated with insufficient metabolism and/or sensitivity of drugs, and vectors comprising such polynucleotides are provided. Furthermore, host cells comprising such polynucleotides or vectors and their use for the production of variant CYP3A4, CYP3A7 and hPXR proteins are described. In addition, variant CYP3A4, CYP3A7 and hPXR proteins and antibodies specifically recognizing such proteins as well as transgenic non-human animals comprising the above-described polynucleotide or vectors are provided. Described are also methods for identifying and obtaining inhibitors for therapy of disorders related to the malfunction of the CYP3A4, CYP3A7 and hPXR genes as well as methods of diagnosing the status of such disorders. Pharmaceutical and diagnostic compositions comprising the above-described polynucleotides, vectors, proteins, antibodies and inhibitors by the above-described method are provided. Said compositions are particularly useful for diagnosing and treating various diseases with drugs that are substrates, inhibitors or modulators of the CYP3A4, CYP3A7 or hPXR gene product.

THIS PAGE BLANK (USPTO)